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No.

Degradation of Aflatoxins in Coconut Oil and Copra meal (poonac)

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(*Paper accepted* : 12 *October* 1976)

Abstract : Coconut is an excellent medium for the growth of aspergilli and for aflatoxin accumulation. Oil derived from mouldy coconut may show high levels of aflatoxin ; even commercial coconut oil from processed copra often shows low or medium levels of aflatoxin. On account of its use as an article of human food, the control of aflatoxin contamination in coconut oil is an urgent problem. The spontaneous drop of aflatoxin levels in stored oil, reported earlier, has been investigated and was found to be due to photodegradation by sunlight, probably through its content of ultraviolet light. Exposure of oil, with either high or low levels of aflatoxin, to sunlight (over 60 cal/cm^2) was found to significantly reduce the levels of aflatoxin. Commercial contaminated oil was decontaminated to aflatoxin levels of less than 30 ppb ; there was no change in the FFA content or colour. It is suggested that exposure to sunlight could form the basis of a cheap and efficient method of the industrial decontamination of aflatoxin contaminated coconut oil in tropical countries.

1. Introduction

The levels of relative humidity and environmental temperature in tropical countries are precisely the conditions which favour fungal colonisation of stored foods, especially those with more than 10% substrate moisture. This results in the accumulation of toxic fungal metabolites including aflatoxin. Coconut is one such source of a human and animal food which has been shown earlier to be an excellent medium for aflatoxin accumulation. The presence of aflatoxigenic strains of *Aspergillus flavus* and of aflatoxins in food products derived from coconut in Sri Lanka, was first reported in 1971.[2]

Aflatoxin was incriminated as the cause of 106 human deaths in an epidemic of poisoning in Western India due to the consumption of mouldy maize[14] and an outbreak of aflatoxicosis occurred among goats in Sri Lanka causing considerable economic losses to goat farming.[19] The cause of the outbreak was found to be aflatoxin which was present in a mouldy coconut-based food concentrate fed to the animals.

In the light of these observations, urgent measures are needed to prevent aflatoxin contamination, or to evolve cheap and effective methods for the detoxification of contaminated substrates, especially in poor tropical countries.

Prevention of fungal colonisation is the rational, cheapest and most efficient step in the control of aflatoxin contamination. The possible industrial use of smoke protection of solid substrates has been described elsewhere.[3] If fungal growth can be prevented during curing and storage, the question of detoxification does not arise.

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However, once the food items are contaminated, they should either be discarded, treated for removal of degradation of aflatoxin or diverted to the manufacture of non-edible products.

The contributions by various authors on the decontamination of aflatoxin-contaminated substrates are numerous and are reviewed by Dollear[9] and by Detroy, Lillehoj and Ciegler.[8] Removal of aflatoxins could be brought about either by the selective removal of the contaminated kernels by mechanical,[10,13] electrical[4] or manual means. Once a batch of kernels or seeds containing a few contaminated ones is processed for oil, the aflatoxins pass into both the oil and pressed cake. At this stage, the toxin will be more uniformly distributed in the oil or cake and its destruction could be achieved only by chemical, thermal, radiation or microbiological treatment.

Chemical inactivation could be brought about by using gases such as chlorine, sulphur dioxide, ammonia, methyl amine, ozone, propylene oxide or by liquids such as hydrogen peroxide, sodium hydroxide, formaldehyde and acids. The extraction of the toxins by various combinations of solvents such as acetone, ethanol, hexane, isopropanol, methanol and water has also been described.[8] The chemical methods have the disadvantage of residual effects from the chemicals used and the production of various toxic compounds due to reactions between components of foods and the inactivating agents.[11] The biological value of the foods, too, could be reduced due to the action of the chemicals or solvents.

Thermal treatment involves roasting or cooking of the substrate before consumption. Aflatoxins are stable to high degrees of dry heat. Roasting[15] and cooking[16] bring about only partial degradation of aflatoxins. Even with autoclaving at 15 psi[5,7] variable results were obtained with regard to the efficiency of heat detoxification. A further disadvantage could be that some vitamins and amino acids in the substrates are destroyed during the heating.

Among the radiation methods, high doses of radiation were found to be necessary to bring about detoxification.[17] At such levels of radiation, the quality of foods could be adversely affected.[12] Ultraviolet radiation has been successfully used in detoxification of aflatoxins. Aibara and Yamagishi[1] have shown that the products of ultraviolet degradation of aflatoxins are less toxic than aflatoxins themselves.

By screening a large number of micro-organisms, Ciegler *et al.*[6] showed that *Flavobacterium aurantiacum* could remove aflatoxin B_1 in solution ; they successfully applied it to milk and butter. However, the possibility of other effects such as lipolysis due to these organisms was not ruled out.

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Some of the methods described above, adapted for the removal or inactivation of toxins, involve the use of costly equipment and chemicals. On the other hand, the methods could cause a loss of nutritive value of the treated food, alterations of flavour and aroma and the presence of residual chemicals. Newly formed by-products of treatment may be of unknown characteristics and potentially hazardous.

Among the various coconut-based food items, copra, coconut oil and poonac were the major items which were found to contain aflatoxins. Fungal colonisation occurs on stored kernels with more than about 10% moisture and the accumulated aflatoxins pass into oil and poonac during processing. No fungal growth occurs in coconut oil but colonisation in poonac occurs only under exceptional conditions of very high moisture and even then only very low aflatoxin concentrations are produced. In a copra heap, only a few kernels will usually be mouldy. The kernels are handled individually by workers during curing, storage and transportation to the crushers and hence they could easily be separated manually, because the fungal growth is visible. Since the contamination of oil and poonac is due to the admixture of a few contaminated kernels, the cheapest and easiest method of control would be the manual separation of mouldy kernels.

The most effective method for detoxifying contaminated oil is chemical refining which removes aflatoxins with pigments. This method, however, is expensive and hence cannot be used for all the oil produced in this country. The chemical removal of aflatoxins, which is the only effective method applicable to poonac, is a costly process.

It has been reported earlier[2] that a spontaneous fall of the aflatoxin content occurs in coconut oil during prolonged storage in the laboratory. This phenomenon was studied as a possible basis for a cheap and effective method for the decontamination of oil and poonac. This paper reports that the degradation of aflatoxin in oil under storage was found to be due to sunlight and that experimentally contaminated oil as well as contaminated commercial oil was effectively decontaminated by exposure to sunlight.

2. Materials and Methods

2.1. Oil

2.1.1. *Preparation of samples :*

Freshly grated coconut was inoculated with a highly aflatoxigenic culture of *Aspergillus parasiticus* NRRL 2999 and the highly toxic oil expelled from the culture was added to commercial coconut oil to produce samples of oil containing 10 ppm to 20 ppm of aflatoxin B_1.

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2.1.2. *Degradation under storage*

Replicate portions of the oil mixtures were treated as follows :—

(*a*) storage at —85°C in total darkness,

(*b*) storage at room temperature (25°C ± 2°C) in total darkness,

(*c*) heated to 70°C (dry heat) and stored at room temperature in total darkness,

(*d*) storage at room temperature in diffuse sunlight (inside a room close to a window excluding direct sunlight).

Samples were withdrawn for assay at 10 day intervals, for 4 months.

2.1.3. *Degradation by sunlight*

Coconut oil samples containing different aflatoxin levels, either experimentally produced or purchased from commercial sources, were exposed in the form of thin layers ($1\frac{1}{2}$ cm or 4 cm) for different periods, to various intensities of sunlight. The amount of sunlight falling within the duration of the exposure, was calculated from readings on a Robitzsch type actinogram.

The 'quality' of the oil before and after treatment was estimated by its free fatty acid (FFA) values and colour.

2.1.4. *Penetration of sunlight into thick layers of oil*

Coconut oil layers of 30 cm thickness in glass cylinders with the sides completely covered with black paper, were exposed to direct sunlight so that the light fell through the top of the layer of oil. Samples withdrawn from the top, middle and bottom were assayed for aflatoxin.

2.1.5. *Effect of dry heat :*

Samples of oil were (*a*) maintained at 100°C or (*b*) heated to 300°C until they showed discolouration.

2.1.6. *Effect of steam.*

Steam was passed into coconut oil maintained at 100°C in a boiling water bath. Any water of condensation was accounted for in the assay.

2.2. Poonac

2.2.1. *Effect of sunlight.*

Powdered samples of poonac were exposed to direct sunlight in the form of a layer $\frac{1}{2}$ cm in thickness, for varying periods.

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2.2.2. *Methods of assay*

All samples were extracted by the aqueous acetone procedure[18] and aflatoxins were estimated on thin layer chromatograms (silica gel 'G Merck') with standards of aflatoxins B_1 and G_1 in chloroform run in methanol : chloroform (3 : 97) and acetone : chloroform (1 : 9). Samples were prepared and assayed in duplicate and mean values from typical experiments are presented.

2.3. Fungal strain and inocula

Samples of grated, fresh coconut were inoculated with *A. parasiticus* NRRL 2999 spores from a 2 week old culture on potato dextrose agar ('Difco') suspended in 0.1% 'Tween 80'. Cultures were shaken manually on alternate days and were steamed on the 7th day before extraction of oil.

3. Results

3.1. Oil

Of the four sets of coconut oil mixtures stored under different conditions only the samples exposed to diffuse sunlight at room temperature, showed a drop in aflatoxin levels to below 0.03 ppm (Figure I). The aflatoxin levels in the other samples showed no significant change.

On exposure of replicates of the same mixtures to direct sunlight in the form of layers of 1 cm and 4 cm in thickness, for different periods, a drop in aflatoxin levels was observed after 1 h to 2 h exposure at midday. Very low aflatoxin values were recorded after exposure to about 100 calories/square cm (cal/cm^2) of sunlight (Figure II).

With naturally contaminated commercial coconut oil samples containing about 0.1 ppm of aflatoxin B_1, degradation of aflatoxin to levels below 0.03 ppm was possible on exposure to 40 cal/cm^2 of sunlight in the form of a layer 4 cm in thickness (Figure III).

In contrast to thin layers, thicker (30 cm) layers of oil showed no reduction in aflatoxin levels, after exposure to sunlight for similar periods.

No changes in aflatoxin levels were observed in coconut oil subjected to dry heat or steam at 100°C (Table 1). Although there was degradation of aflatoxin on dry heating at 300°C, the loss was partial and the treated oil yet contained aflatoxin above permissible levels (Table 2).

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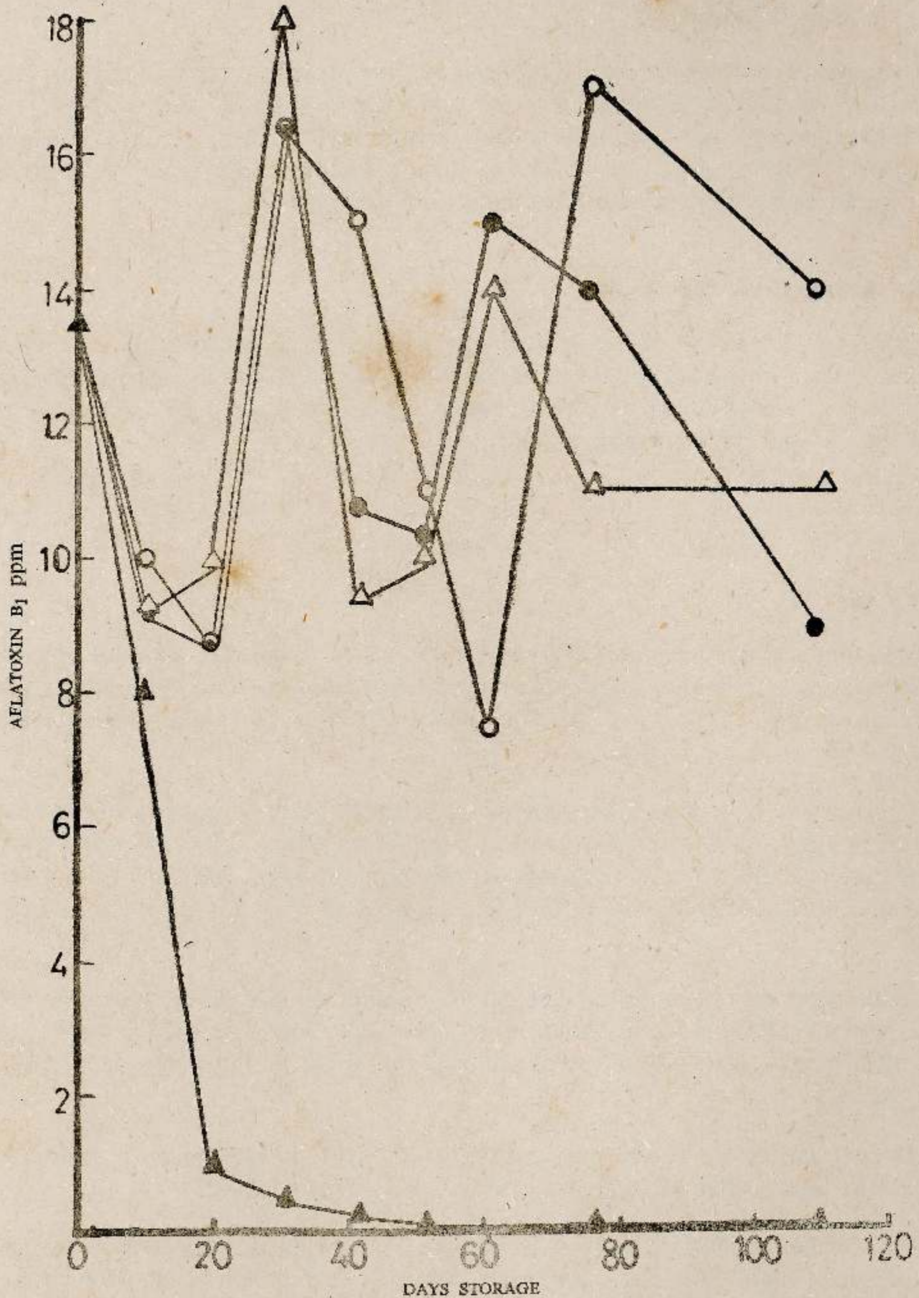

Figure I. Effect of storage at —85°C in the dark ●—●; storage at 25°C ± 2°C in the dark ○—○; storage at 25°C ± 2°C in the dark after heating at 70°C for ½ hr Δ—Δ; and storage at 25°C ± 2°C in diffuse sunlight ▲—▲; on the aflatoxin B_1 content of experimentally contaminated coconut oil. Aflatoxin content in parts per million (ppm).

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Figure II. Effect of exposure to sunlight, on the aflatoxin B_1 content of experimentally contaminated coconut oil containing high levels of toxin. ●—● = replicate samples.

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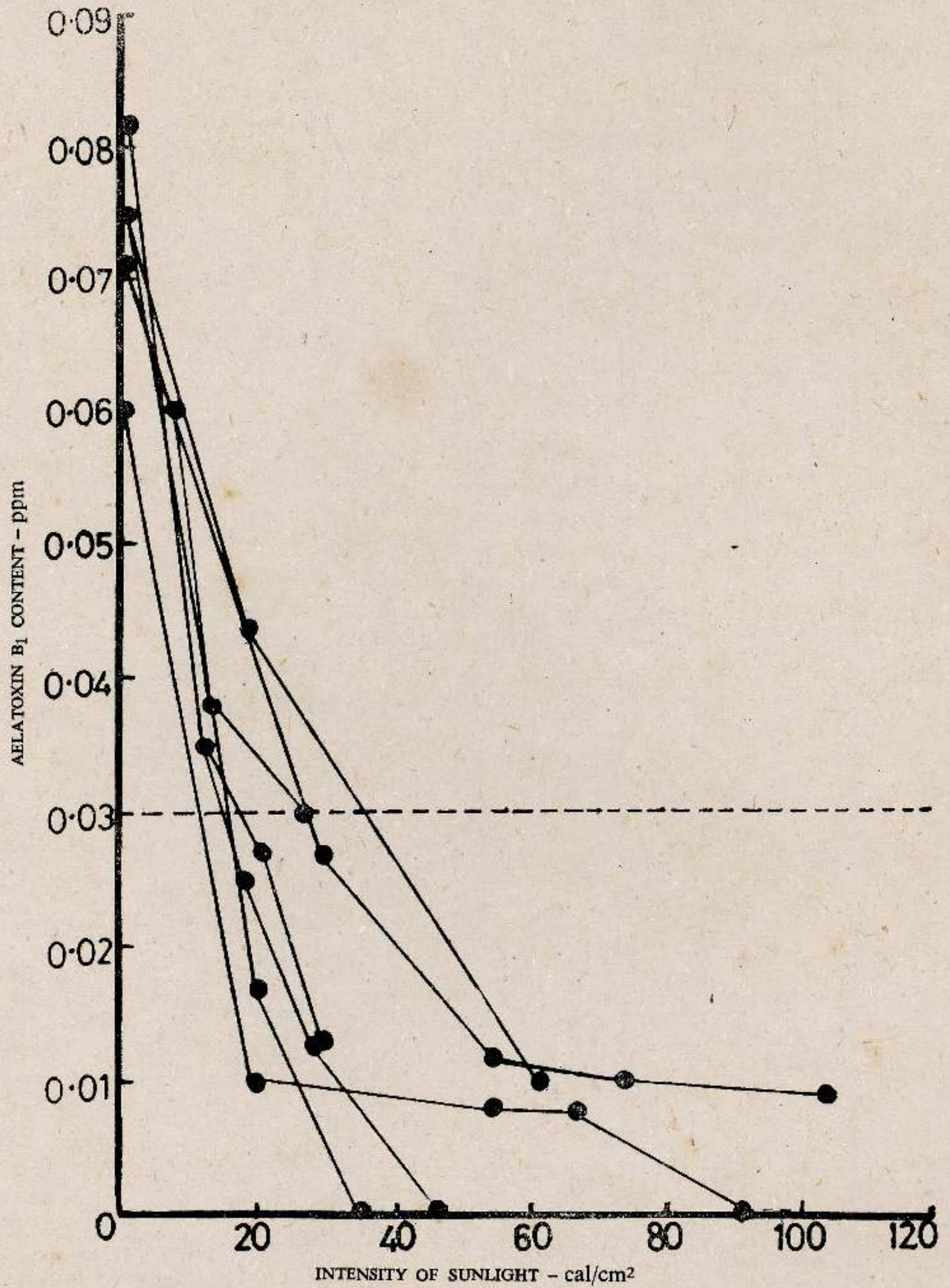

Figure III. Effect of exposure to sunlight, on the aflatoxin B_1 content of commercial coconut oil containing low levels of toxin. ●—● = replicate samples.

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TABLE 1. The effect of dry heat at 100° C and steam at 100°C on the content of aflatoxin in experimentally contaminated coconut oil. Duration of heating 1h.

Experiment number	*Aflatoxin B_1 (ppm)*			*Aflatoxin G_1 (ppm)*		
	before treatment	*dry heated*	*steam heated*	*before treatment*	*dry heated*	*steam heated*
1	5.0	4.4	2.5	1.0	0.9	0.9
2	1.3	1.0	1.3	0.8	1.0	1.0
3	0.9	0.9	0.7	0.2	0.1	0.1

TABLE 2. The effect of dry heat on the content of aflatoxins in coconut oil.

Treatment	*aflatoxin content (ppm)*			
	B_1		*G_1*	
	before treatment	*after treatment*	*before treatment*	*after treatment*
10 min at 180°C to 215°C	8.5	5.0	4.3	1.5
10 min at 300°C	0.20	0.06	0.05	0.03

After photodegradation of aflatoxins, no new fluorescent compounds were observed in oil which was almost totally detoxified. On two occasions when partial decontamination occurred, a reddish purple compound of the same R_f as aflatoxin B_1 together with a violet blue compound of a slightly higher R_f were seen on TLC run in methanol : chloroform.

3.2. Poonac

No significant changes in aflatoxin levels were seen on exposure of powdered poonac to direct sunlight for upto 3½ h, in a layer of 5 mm thickness.

4. Discussion

Of the various laboratory methods described in the literature for the degradation of aflatoxins in agricultural products, only chemical treatment appears to have been successful. However, the disadvantages of these methods are (1) the removal or conversion into non-availablity of essential components in foods, for example, lysine,[20] (2) chemical reactions leading to the formation of secondary toxic products.[11] There is no literature on the successful adaptation of any of these methods, on an industrial scale for the detoxification of contaminated agricultural products.

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Although aflatoxins have been the subject of very extensive research and in spite of the fact that photodegradation of aflatoxin was known since their discovery, no work has, as far as we are aware, been done to utilise natural photodegradation as the basis of a detoxification method for aflatoxins in oil. This is probably due to the fact that most of this research has been done in temperate countries where sunlight is of low intensity and is not uniform throughout the year. On the other hand, the ready availability of chemicals and technology may have diverted research interests to the use of chemicals in detoxification of aflatoxins. Under tropical conditions, the sunlight falling during the day is uniform and of high intensity over a large part of the year. A method which uses sunlight would be inexpensive and practicable with minimal technical complexity, features which would be of advantage in poor, tropical countries such as ours.

Our results suggest that the decontamination of coconut oil was due to photoactivity rather than to aflatoxin degrading enzymes. Aflatoxin is well known to be degraded by ultraviolet light, and it may be this component in sunlight that was responsible for the degradation of aflatoxin in the oil. The direct heating effect of sunlight, causing a rise of temperature by about 10°C to 15°C was probably a negligible factor in this degradation, since even much higher degrees of dry heat were ineffective.

The absence of a change in the FFA content and colour of the exposed oil indicates that exposure to sunlight could be an acceptable method in industrial practice. In the application of this method under field conditions, the amount of sunlight available throughout the year and the penetration of light into thick layers of oil in tanks are important factors. Our results indicate that a sunlight intensity of approximately 60 cal/cm^2 was sufficient to bring down the aflatoxin levels to below 0.03 ppm (the maximum permissible level for aflatoxins in human foods proposed by the Protein Advisory Group of the WHO/FAO/UNICEF). The sunlight data recorded for the year 1973 at Peradeniya is shown in Table 3.

TABLE 3. Solar radiation data for Peradeniya in 1973.

	cal/cm^2
mean per day	353.7
range per day	92*b*—532

mean values recorded at different hours of the daytime.

8.00 am — 9.00 am	40
9.00 am — 10.00 am	50
10.00 am — 11.00 am	60
11.00 am — 12 noon	90
12 noon — 1.00 pm	100
1.00 pm — 2.00 pm	80

b = on a rainy day.

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Most of the coconut mills in Sri Lanka, are situated in the Negombo and Colombo districts which experience less rainfall than Peradeniya. Hence the duration of sunlight in these mills will be greater than in Peradeniya. Thus, it may be possible to make use of sunlight for the detoxification of aflatoxin in coconut oil under industrial conditions in the mills in these areas.

Since oil is stored in overhead tanks for several days after processing, it may be convenient to use a system which permits the oil to flow under gravity into storage tanks, along narrow transparent glass pipes or in shallow layers in trays, while being exposed to sunlight, for about one hour in transit. This method will involve only the initial cost of setting up the flow system. Further work will however be necessary for the adaptation of this method under field conditions.

Although no fluorescent products were detected after photodegradation of aflatoxins in coconut oil, further work will be needed on the toxicity of the treated oils, since the properties of photodegraded oils have not hitherto been investigated.

Since coconut oil is used for the frying of foods it was of interest to know of the fate of aflatoxins in oil at high temperatures. The stability of aflatoxins in oil at 100°C with only partial degradation even at 300°C, at which temperature the oil becomes discoloured, indicated that contaminated oil is unsuitable for cooking even at high temperatures.

With poonac particles, efficient degradation of aflatoxins was not achieved by exposure to sunlight ; this was probably because the light did not penetrate the particles. Exposure to ultraviolet light was also found to be unsuccessful in reducing the toxicity of aflatoxin contaminated peanut meal.[11] Methods involving autoclaving were not attempted since the food values of such heated products may have been altered by the heat treatment, as has been described of groundnuts.[20] However, the efficiency of steam treatment with powdered copra as practised in certain mills, before oil extraction, merits study.

Further experiments in the field and at industrial oil processing mills will be needed to define the exact conditions under which degradation of aflatoxin in oil could be achieved, in industrial practice.

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A Comparative Study of the Geochemistry of Arsenic, Antimony and Bismuth in Minerals from a Fractionated Sequence

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(*Paper accepted :* 15 *October* 1976).

Abstract : A comparative study of the group V elements As, Sb, and Bi in minerals from a complete fractionation sequence as exemplified by the Skaergaard intrusion reveals that their geochemical behaviour is governed chiefly by their chemistry. Arsenic shows a predominance of the As^{5+} ion in the magma. In the minerals, it exists both as As^{3+} and As^{5+} substituting for other cations of suitable size and charge. The tendency of arsenic to form complex oxyions enables it to act as a network former in silicates. It acts as a network modifier when free ions are formed. This dual behaviour characterizes the geochemistry of arsenic. Antimony on the other hand shows a $\frac{Sb^{5+}}{Sb^{3+}}$ ratio varying from 0.05 to 0.2 indicating the predominance of the Sb^{3+} ion. It is very likely that it is mainly the Sb^{3+} ion which substitutes for major element cations of similar ionic radius even though some pentavalent antimony may form complex ions and become enriched in the residual liquid. The last of the group V elements, bismuth, shows no Bi^{5+} ion in nature, its geochemistry being entirely that of the Bi^{3+} ion. Bismuth prefers to enter the oxide minerals such as magnetite and ilmenite as against the silicates, and substitutes mainly for the Fe^{2+} ion. Accordingly, bismuth silicate minerals are extremely rare while bismuth oxide minerals are common.

1. Introduction

The geochemistry of the group V elements As, Sb and Bi is not well known due to the dearth of information regarding their distribution in rocks and minerals. Much of the earlier data obtained by colorimetric, polarographic and spectrographic techniques,[2,3,4,13,16,19] can be considered as only being semi-quantitative since the detection limits of these techniques for As, Sb and Bi are relatively higher when compared to their abundance in rocks and minerals. Very few analyses by the precise and highly sensitive analytical techniques such as neutron activation analysis and isotope dilution analysis have been made.[6,8,11,12,17,18]

This work deals with a comparative study of the group V elements in the minerals of the Skaergaard intrusion of East Greenland (Figure 1) by far the best testing grounds for theories on trace element behaviour.[21] Since the publication of the classic paper on the distribution of trace elements in the Skaergaard intrusion by Wager and Mitchell,[22] which included the study of 18 trace elements, more work on the geochemical distribution of some elements not hitherto studied has been carried out. A total of more than 60 elements have been now studied from the point of view of their geochemical distribution, thereby making the Skaergaard intrusion the best documented layered igneous complex. This study highlights the behaviour of As, Sb and Bi in the minerals as influenced by their chemical properties.

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2. Experimental

2.1 Arsenic and Antimony

As and Sb in the Skaergaard samples have been determined by Esson *et al.*[8] using modifications of neutron activation methods.[17,18] Briefly, the procedure is as follows:—

100 mg portions of the powdered rocks and minerals are irradiated in a nuclear reactor for 3 days at a flux of about 10^{10} neutrons/cm^2 sec along with 10 mg portions of As_2O_3 and 0.1 g to 0.2 g portions of a dilute Sb solution (10 μ g Sb/g of solution) to serve as standards. After irradiation, the samples together with 50 mg amounts of As_2O_3 and Sb_2O_3 carriers are decomposed by sintering with 2 g Na_2O_2 in nickel crucibles at 490°C ± 10°C. The samples and standards are then subjected to a chemical separation process,[17] finally the As and Sb being precipitated as sulphides using thioacetamide. The activities of the final precipitates are measured and the radiochemical purity checked by decay curves.

2.2 Bismuth

Bismuth in the Skaergaard samples were determined by Dissanayake,[6] using sub-stoichiometric isotope dilution analysis.[10,15]

Approximately 100 mg samples are decomposed with a HF—$HC1O_4$ mixture spiked with Bi^{207} tracer. The bismuth is converted to the iodide and extracted with methyl isobutyl ketone. The extract is washed with acidiodide and the bismuth brought into the aqueous phase before being complexed with a known sub-stoichiometric amount of EDTA. The excess bismuth is then extracted as the iodide and the Bi^{207} in the aqueous phase EDTA complex counted and the specific activity determined. The bismuth contents of the samples are derived using a calibration curve obtained by carrying standard bismuth solutions through the same procedure as the samples.

3. Results and Discussion

3.1 The Chemistry of As, Sb and Bi

Before considering the comparative behaviour of As, Sb and Bi, it would be profitable to outline some of their more important chemical properties as they could influence the gross geochemical behaviour (Table 1).

From the above table of properties it can be seen that while arsenic and antimony form the M^{5+} ion, the Bi^{5+} ion is not known in nature. The tendency to form compounds with electropositive elements decreases markedly from arsenic to bismuth. Similarly, the covalent nature of bonds decreases down the group with bismuth showing increased cationic behaviour. In the +5 state all three elements are predominantly non-metallic and acidic but as this state is less readily attained as

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the atomic weight increases from As to Bi, there is a marked decrease in the acidic character down the group as shown by the fact that bismuth forms the most basic compounds. The tendency to form oxyanions decreases from As to Bi with the latter forming no oxyanions in nature.

TABLE 1. Chemical data for arsenic, antimony and bismuth.

	As	Sb	Bi
Outer electronic configuration	$3d^{10}\ 4s^2\ 4p^3$	$4d^{10}\ 5s^2\ 5p^3$	$5d^{10}\ 6s^2\ 6p^3$
Sum of the first three ionization potentials ev	59.0	52.3	52.0
Electronegativity	2.20	1.82	1.67
Ionic radii[24]	As^{5+} (0.42°A)	Sb^{3+} (0.85°A) Sb^{5+} (0.69°A)	Bi^{3+} (1.10°A)
Covalent radii (for trivalent type)	1.21	1.41	1.52
Main oxidation states	+3 +5	+3 +5	+3
Oxides formed	As_4O_6, As_2O_5	Sb_4O_6, Sb_2O_5	Bi_2O_3
Heat of vaporization kgcal/g atom	7.75	46.6	42.7

3.2 The behaviour of As, Sb and Bi in the Skaergaard fractionation

Figure 2 shows the variation of As, Sb and Bi during fractionation. Arsenic shows a marked increase in the last stages of the fractionation sequence, indicating its tendency to remain preferentially in the liquid. Antimony and bismuth, too, show somewhat similar curves even though the concentration of arsenic relative to antimony changes from about 1 to 5 during progressive fractionation. Bismuth, on the other hand, does not show an enrichment in the last stages of fractionation as arsenic, even though it is greater than that of antimony. This relative variation of the three elements during increasing solidification of the magma is illustrated in Figure 2.

It can be seen that both liquid and solid trends are very similar. The liquid trend observed for As, Sb and Bi shows an order of enrichment as follows :—

0% to 40% solidification	: As > Bi > Sb
40% to 80% solidification	: Bi > Sb > As
80% to 100% solidification	: As > Sb > Bi

Figure 1 shows the minerals present and their compositions in the fractionation sequence. The early liquid is rich in Sb and as stated by Esson *et al.*,[8] it becomes concentrated in the early magnesian olivines and the amount of antimony in the olivines decreases as differentiation causes a progressive change in the olivine composition from Fo_{70} to Fo_2. In the case of bismuth, there seems to be very little tendency to enter the early formed minerals.

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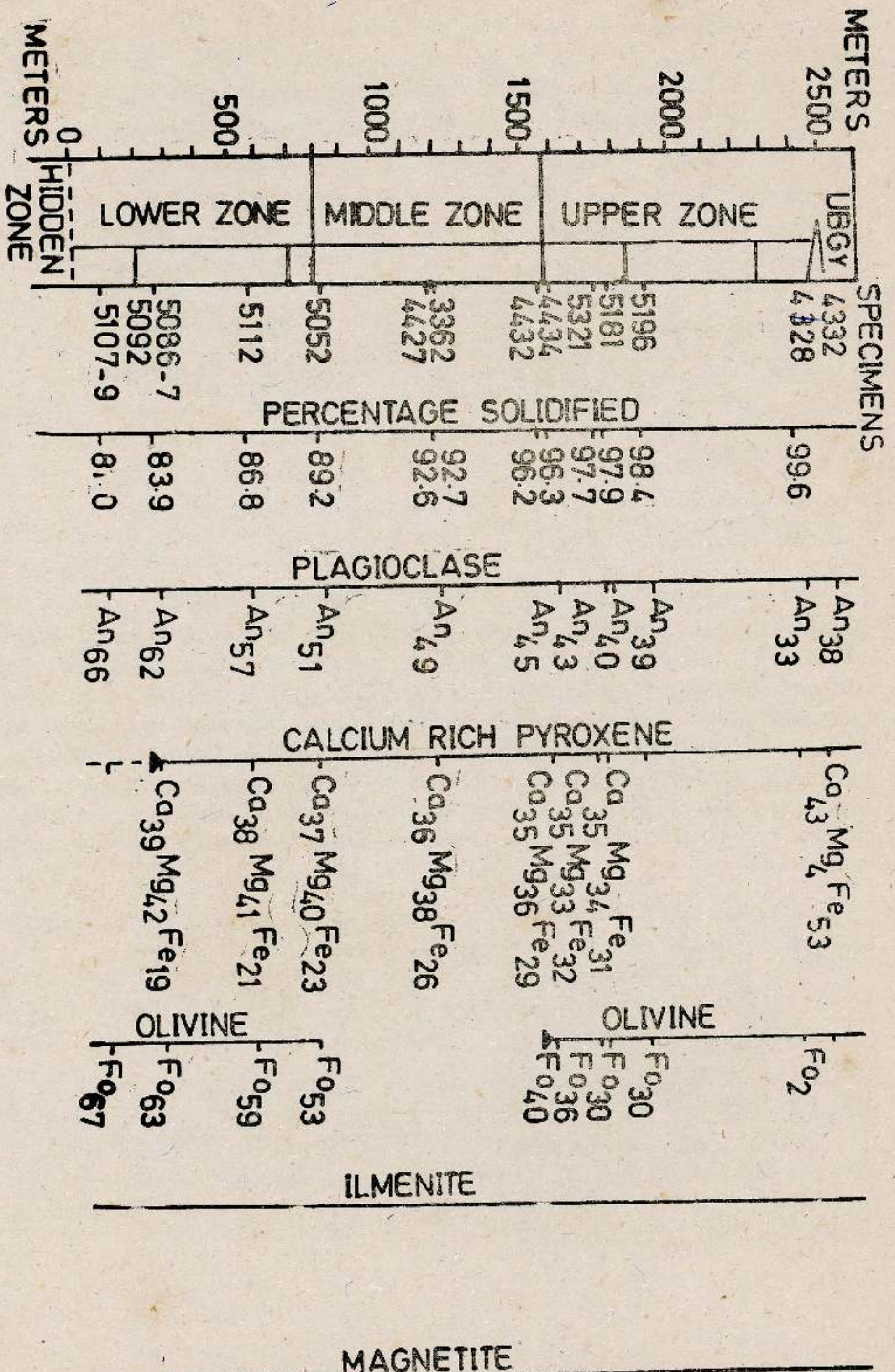

Figure 1. Minerals present in the various rocks of the Layered Series of the Skaergaard Intrusion. Discontinuous vertical lines relate to intercumulus minerals or those of indeterminate status.

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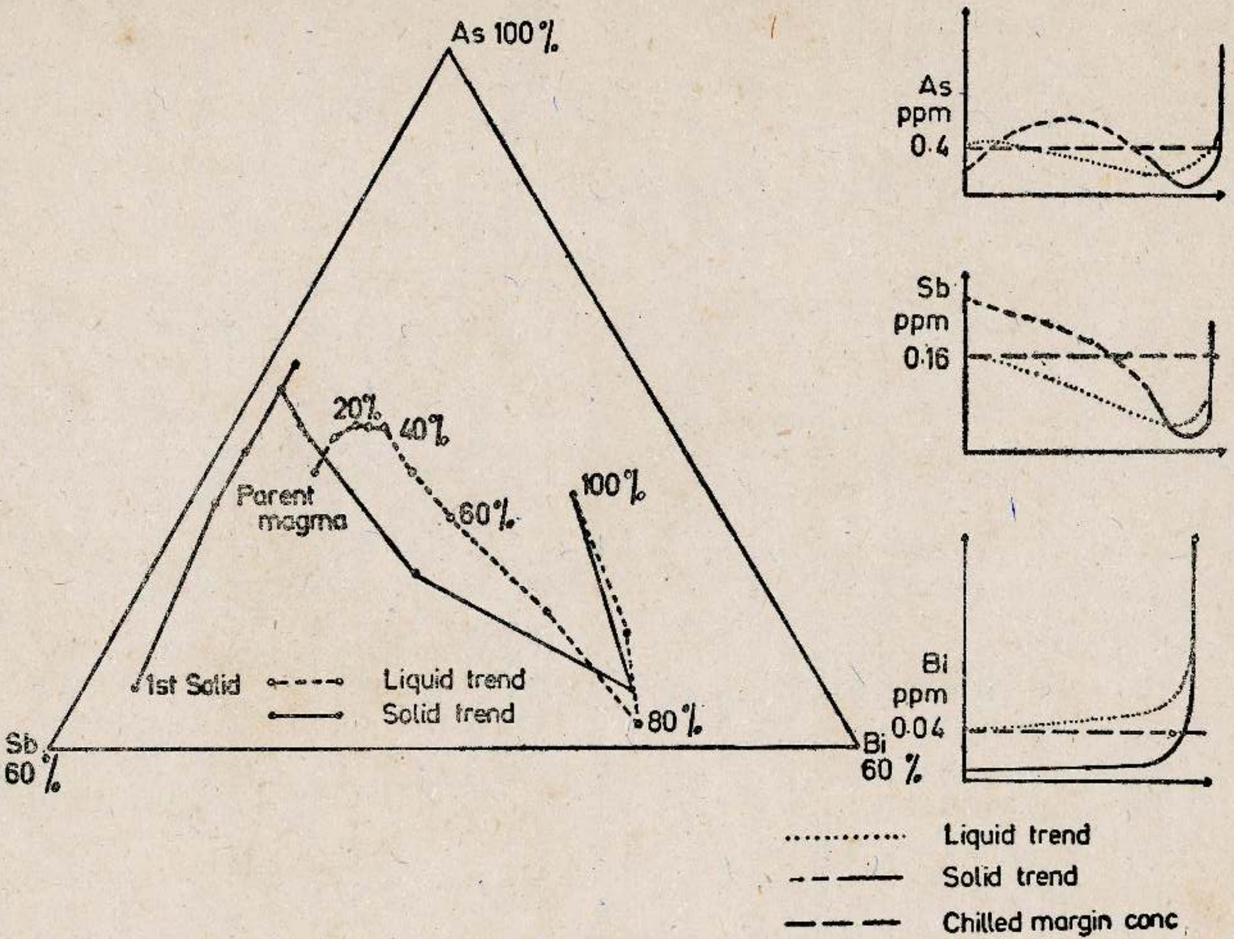

Figure 2. The relative distribution of arsenic, antimony and bismuth.

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From about 40 % to 80 % solidification a distinct trend towards the bismuth corner is seen (Figure 2). While the antimony concentration changes only within narrow limits, arsenic shows a rapid decrease in both solid and liquid. Thus, it is at about 40% solidification that bismuth shows signs of incorporation in the solid corresponding to the enrichment in the liquid.

In the last 20% of solidification, arsenic once again shows the highest enrichment in the liquid. This is mainly due to its strong tendency to remain in the liquid rather than entering the iron rich silicate and oxide mineral phases. The arsenic contents of the minerals contribute little to the total arsenic content of the rocks, the rest being contributed by the trapped liquids. Further, arsenic shows a greater tendency than antimony and bismuth to enter the minor iron sulphide phase which appears in the last stages of the fractionation sequence. At these stages, Sb and Bi show a very similar trend, both elements tending to enter the primary minerals rather than remaining in the interstitial liquid as As does. Thus in the final stages of fractionation the order of enrichment in the liquid is As > Sb > Bi. It is of interest to note that this order is the same as the order for the tendency to form complex oxyanions which tend to accumulate in the late stage liquids. While the lithophile tendency increases in the order Bi > Sb > As, the chalcophile tendency increases in the reverse order.

3.3 Distribution of As, Sb and Bi in minerals

Tables 2 and 3 illustrate the As, Sb and Bi contents of the minerals separated from rocks. The entry of As, Sb and Bi into the cationic sites of the rock forming minerals can best be discussed in the light of the theory of Ringwood.[14]

Ringwood[14] considers 2 groups of ions. (*a*) Free ions which act as network modifiers, and (*b*) complex ions which act as network formers. According to him, the group into which any ion falls depends on the affinity of that ion for oxygen as governed by its ionic potential $\frac{\text{(cationic changes)}}{\text{(ionic radius)}}$. Accordingly, ions with low ionic potential (less than 2.7) enter cationic sites in the structure thereby becoming network modifiers while those with high ionic potential (greater than 4.8) form the centres of complex oxyions and are network formers. Examples of the latter type are

Be^{2+}, B^{3+}, Al^{3+}, Si^{4+}, Ti^{4+}, Zr^{4+}, P^{5+}, As^{5+}, Ta^{5+}, Mo^{6+},

etc.

Between these two groups there is an intermediate group of ions which maintain an equilibrium between complex and non-complex positions. Generally, these are present as free ions in the magma until when the pegmatitic stage is reached these intermediate ions form complexes with the volatile elements not readily accepted into the silicate structures, thereby accumulating in the residual liquids.

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TABLE 2.* Arsenic and Antimony in Constituent Minerals of the Rocks 5181, 5052 and 5086

Rock No. and Description	*Mineral*	*Modal % by weight*	*As PPM*	*As content contributed to rock*	*Sb PPM*	*Sb content contributed to rock*
5181	Plagioclase An_{40}	54.4	0.019	0.0103	0.23	0.1278
Hortonolite	Pyroxene $Ca_{35}Mg_{34}Fe_{31}$	21.9	0.066	0.0145	0.10	0.0230
ferrodiorite	Magnetite	4.5	0.20	0.0095	0.082	0.0045
	Ilmenite	5.5	0.11	0.0061	0.27	0.0121
	Olivine Fo_{30}	13.2	0.17	0.0224	0.20	0.0271
				0.0628		0.1945
	Whole rock (average)	0.17			0.17	
	Fine dust from mineral separation		0.24		0.069	
5052	Plagioclase An_{51}	42.2	0.027	0.0114	0.032	0.0135
Middle gabbro	Pyroxene $Ca_{37}Mg_{40}Fe_{23}$	29.4	0.048	0.0141	0.026	0.0076
	Magnetite	9.5	0.089	0.0086	0.025	0.0024
	Ilmenite	14.6	0.021	0.0031	0.150	0.0212
	Olivine Fo_{53}	3.3	0.086	0.0029	1.17	0.0386
				0.0401		0.0833
	Whole rock (average)	0.091			0.046	
	Fine dust		0.11		0.042	
5086	Plagioclase An_{62}	46.1	0.021	0.0097	0.036	0.0166
Lower Olivine	Pyroxene $Ca_{47}Mg_{34}Fe_{19}$	25.6	0.107	0.0256	0.032	0.0081
gabbro	Magnetite	1.8	3.6	0.0648	0.11	0.0017
	Olivine Fo_{53}	25.7	0.074	0.0193	1.37	0.3500
				0.1194		0.3764
	Whole rock (average)	0.22			0.14	
	Fine dust		0.29		0.12	

* After Esson *et al.*

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TABLE 3. Bismuth in Minerals of the Skaergaard Intrusion

Mineral	*Modal wt.%*	*Bi (ppm) Replicate analyses*		*Average Bi (ppm)*	*Contribution to whole Bi (ppm)*	*% Contribution to whole rock B*
5181 *Upper Zone a* (UZa)						
Plagioclase An_{40}	54	0.17	0.14	0.15	0.08	28.5
Pyroxene $Ca_{55}Mg_{34}Fe_{31}$	23	0.13	0.15	0.14	0.03	10.7
Olivine Fa_{70}	13	0.32	0.39	0.35	0.05	17.8
Magnetite	4.5	0.38	0.32	0.35	0.01	3.5
Ilmenite	5.5	0.46	0.46	0.46	0.02	7.1
					0.19	67.6
				Whole rock	0.28	
4427 *Middle Zone* (MZ)						
Plagioclase An_{49}	26.7	0.03	0.04	0.04	0.01	$7.1\frac{2}{3}$
Pyroxene $Ca_{36}Mg_{38}Fe_{26}$	36.0	0.04	0.03	0.04	0.01	7.1
Magnetite	12.4	0.28	0.21	0.24	0.03	21.4
Ilmenite	22.6	0.27	$0.23\frac{2}{3}$	0.25	0.06	42.8
					0.11	78.4
				Whole rock	0.14	
5052 *Middle Zone* (MZ)						
Plagioclase An_{51}	48.0	0.06	0.05	0.06	0.03	18.7
Pyroxene $Ca_{37}Ma_{40}Fe_{23}$	29.0	0.05	0.05	0.05	0.01	6.2
Magnetite	9.0	0.32	0.35	0.34	0.03	18.7
Ilmenite	14.0	0.28	0.24	0.26	0.04	25.0
					0.11	68.6
				Whole rock	0.16	
5112 *Lower Zone* (LZb)						
Plagioclase An_{57}	37.2	0.10	0.10	0.10	0.04	28.5
Pyroxene $Ca_{/23/}Mg_{41}Fe_{21}$	45.0	0.08	0.07	0.08	0.03	21.4
Olivine Fa_{43}	14.7	0.09	0.10	0.09	0.01	7.1
					0.08	57.0
				Whole rock	0.14	
5092 *Lower Zone* (LZb)						
Plagioclase An_{62}	19.5	0.06	0.05	0.06	0.01	7.6
Pyroxene $Ca_{39}Mg_{42}Fe_{19}$	31.2	0.04	0.08	0.06	0.02	15.3
Olivine Fa_{37}	47.8	0.11	0.14	0.12	0.06	46.1
				Whole rock	0.09	69.0
4507* *chilled marginal gabbro*						
Plagioclase An_{72}	51.3	0.10	0.12	0.11		
Pyroxene $Ca_{37}Mg_{40}Fe_{23}$†	27.9	0.17	0.10	0.13		
Olivine Fa_{41}	19.0	0.12	0.13	0.13		
4314 Apatite		0.09	0.08	0.09		
5275 Pyrrhotite		0.41	0.38	0.40		

*Represents 'parent' magma

†Not pure.

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The case of arsenic is interesting if one considers the following criteria for the diadochy between trace element complex ion and the tetrahedral complex ion SiO_4^{4-}.[14]

(*a*) The substituting ion must be of tetrahedral configuration ; any others will tend to accumulate in residual liquid fractions.

(*b*) The central cation in the complex must have a radius close to that of Si^{4+}.

(*c*) The ionic potential of the central cation should not be higher than that of Si^{4+} since the cation—oxygen bond would then have a greater covalent character than the Si—O bond. This makes substitution unlikely. A good example is tetrahedral PO_4^{3-} which is never known to substitute for SiO_4^{4-} due to the former having a much higher ionic potential.

Arsenic shows the dual behaviour of a network former and a network modifier.

(a) In pyroxene :

Pyroxene consists of parallel straight chains of SiO_4^{4-} tetrahedra, the oxygens being shared to form repeating units of $(Si_2O_6)^{4-}$, the adjacent chains being linked mainly by cations such as Ca^{2+}, Mg^{2+} and Fe^{2+} even though Fe^{3+}, Al^{3+}, Na^{+}, and Ti^{4+} may also be found to some extent. Since the latter group of ions are known to occur in the pyroxene structure, it is possible for As^{3+} to enter the sites, thereby occupying a network modifying position. The Mg^{2+} and Fe^{2+} ions are mostly in tetragonally distorted octahedral coordination (M_1 position) and the Ca^{2+} ions are surrounded by 6 to 8 oxygens (M_2 position) irregularly. Arsenic will enter only the M_1 position since it cannot enter the M_2 position by substituting for the larger Ca^{2+} ions.

(b) In feldspar :

Here the structure consists of a 3 - dimensional network of SiO_4^{4-} and AlO_4^{5-} tetrahedra, all the oxygens being shared. Cations Na^{+}, K^{+}, Ca^{2+} within the structure maintain electrical neutrality. Since As^{3+} and As^{5+} are too small to substitute for the larger Na^{+}, K^{+} and Ca^{2+} ions, arsenic could enter the network as AsO_4^{3-} groups replacing SiO_4^{4-} tetrahedra. This seems reasonable since like the SiO_4^{4-} group, AsO_4^{3-} is tetrahedral in configuration and has an effective radius of 2.48°A as for SiO_4^{4-}. Further, the radii of the central cations As^{3+} and As^{5+} are similar to that of Si^{4+} and also the degree of covalent character in the bonds.

Thus arsenic could enter the structure of a silicate mineral as a network former and as a network modifier.

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As shown in Table 2, arsenic shows no very marked preference for light or dark minerals. The concentrations of arsenic in the various mineral phases are mainly of the same order of magnitude except for the magnetite, ilmenite and olivine from the latest of the three rocks, i.e. 5181. While there is an increase in the arsenic content of magnetite, ilmenite and olivine with increasing differentiation, it seems to have had no effect on the As contents of plagioclase and pyroxene. Only about half the arsenic content appears to be in the interior of the silicate and oxide minerals, the remainder being in the interstitial material and the outer zones of crystals, much of which is usually lost during mineral separation.

Considering the ionic radii and changes, one could expect the following substitutions:

(*a*) As^{3+}(0.58°A), As^{5+}(0.42°A) for Al^{3+}(0.47°A).

(*b*) As^{3+} for Fe^{3+} (0.57°A) and Ti^{4+}(0.61°A).

(*c*) As^{5+} for Si^{4+} (0.34°A).

Onishi and Sandell[13] assume that the amount of As^{5+} is low, particularly when the $\frac{Fe^{2+}}{Fe^{3+}}$ ratio is high. Experimental work[1,5] on silicate glasses however shows that the +5 state of arsenic is by far the predominant form in glass melts even when the $\frac{Fe^{2+}}{Fe^{3+}}$ ratio is high. It is of interest to note that some recent work on the activities of trace elements in silicate melts[9] shows that there is an increased stability of the higher oxidation states of altervalent trace elements in silicate melts with increasing basicity.

If it is assumed that activities are more or less proportional to concentrations even in the magmatic environment, the oxidation potential of the liquid is given by

$$Eh = -E^\circ + \frac{RT}{nF} \ln \frac{Fe^{3+}}{Fe^{2+}}$$

where E° (for $Fe^{2+} = Fe^{3+} + e$) = — 0.771 V
R = 8.314 absolute joules per degree
T = 1273°K
n = 1 (number of electrons transferred in reaction)
F = 96500 C

Substituting for the Fe^{3+}/Fe^{2+} ratio for the UZb liquid of the Skaergaard Layered Series (Figure 1)[21] we obtain Eh — 0.555 V. On substituting the E° value for the reaction $As^{3+} = As^{5+} + 2e$ in the equation, one arrives at $\frac{As^{5+}}{As^{3+}} \simeq 2$ indicating the predominance of the pentavalent state over the trivalent state. It is of interest to note that in the case of mercury, $Hg \simeq 10^2\ Hg^{2+}$,[7] and for gold, $Au \simeq 10^4\ Au^{+}$.[20]

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Considering the individual minerals, arsenic can only enter the plagioclase structure by the substitution of As^{3+} and/or As^{5+} for Si^{4+} and Al^{3+} in tetrahedral sites, or as As^{3+} in octahedral sites. Esson *et al.*[8] think however, that both the entry of As^{3+} into octahedral and As^{5+} into tetrahedral sites would be unfavourable to the structure.

The fact that pyroxene had 2 to 5 times as much arsenic as the plagioclase from the same rock could be explained in terms of the greater number of sites available for substitution.

As^{3+} for Mg^{2+}, Al^{3+}, Fe^{3+} and Ti^{4+}; octahedral sites.
As^{5+} for Si^{4+} and Al^{3+} ; tetrahedral sites.

In the case of opaque oxide minerals, ilmenite and magnetite, the arsenic found in the structure probably resulted from a straightforward substitution of As^{3+} for Ti^{4+} and Fe^{3+}, respectively. In olivine, substitution of As^{5+} for Si^{4+} is also a possibility. The work of Esson *et al.*[8] also indicates that AsO_4^{3-} probably increases more strongly in the last liquids than As^{3+} showing that the tendency for As^{3+} to replace small cations is greater than the tendency for AsO_4^{3-} to enter the silicate structures in place of SiO_4^{4-}.

In contrast to arsenic, the $\frac{Sb^{5+}}{Sb^{3+}}$ ratios for the Skaergaard magmas vary from 0.05 to 0.2 indicative of the fact that Sb^{3+} is the predominant ion in the basic magmas. It is important to bear in mind the greater stability of the trivalent ion in group V as one moves down from As to Bi. Unlike arsenic, antimony (Sb^{5+}) is unable to form tetrahedral oxyions such as SbO_4^{3-} due to the larger size of Sb^{5+} not being conducive to the formation of small stable coordination groups with oxygen, even though pentavalent groups may form complex ions such as $(Sb_2O_6)^{2-}$ $(SbCl_6)^-$ $(SbF_6)^-$ etc, which generally accumulate in the residual magma. Further, the higher charge of Sb^{5+} would hinder the simple substitution of Sb^{5+} for Mg^{2+}, Fe^{3+}, etc., even though the ionic radii may be similar. Therefore, it is very likely that it is mainly the Sb^{3+} ion which substitutes for major element cations of similar ionic radius even though a little pentavalent antimony may form complex ions and become enriched in the residual liquid.

In the Skaergaard samples, the amount of Sb in olivines seems to decrease steadily as differentiation causes a progressive change in olivine composition from Fo_{70} to Fo_2. Ilmenite is the next favourable host, the substitution presumably being Sb^{3+} for Fe^{2+}. The same explanation can be held for pyroxene and magnetite. In the case of plagioclase it is likely that Sb^{3+} enters octahedral sites normally occupied by Al^{3+}.

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The discussion thus far highlights a decreasing tendency for

(*a*) complex ion formation,

(*b*) M^{5+} formation as against M^{3+} formation,

(*c*) entering the structure as a network former,

as one moves from As to Sb with increasing atomic weight. Accordingly, one could predict that bismuth should almost entirely be Bi^{3+} and that its tendency for complex oxyion formation should be negligible. This is in fact observed. Neither does Bi^{5+} exist in nature nor does bismuth form a complex ion capable of replacing SiO_4^{4-} tetrahedra. Hence, the geochemistry of bismuth is almost entirely that of the Bi^{3+} ion. If this be the case, the only sites available for Bi^{3+} substitution would be the cation sites linking the tetrahedra in silicates and the normal cation sites in the oxide minerals. Since the substituted cations in silicates and oxides (mainly Fe^{2+}) do not show any great disparity as regards their coordination, it seems logical that the preferential entry of bismuth into the oxides is related to the presence or absence of Si^{4+}, O^{2-} or SiO_4^- tetrahedra. It appears that the explanation lies on a possible tendency for Bi^{3+} to establish links with oxygen, the ease of which seems obviously to be hampered by the presence of Si^{4+} which would compete for the O^{2-} ions, In both magnetite and ilmenite, Fe^{2+} positions are more suitable than Fe^{3+} and Ti^{4+} for the linking with O^{2-} ions due to the preferred size of the former. It is also of importance to bear in mind that bismuth is the most electropositive element in group V. The 'oxide preferring' property of bismuth as against a 'silicate preferring' tendency is illustrated by the fact, while bismuth silicate minerals are very rare (the only examples being eulytite ($Bi_4(SiO_4)_2$) and bismutoferrite ($BiFe_2(SiO_4)_2(OH)$)), there are numerous oxide compounds with bismite (Bi_2O_3) as one of the commonest bismuth minerals. An interesting feature in the bismuth silicate mineral eulytite is the occurrence of BiO_6 groups.

Since the geochemistry of bismuth is almost completely that of the Bi^{3+} ion, it is of interest to discuss in detail how the Bi^{3+} ion enters cationic sites in the rock forming minerals as exemplified by those from the Skaergaard intrusion.

Table 3 shows the bismuth contents of the minerals separated from rocks of the Lower, Middle and Upper Zones of the fractionation sequence. It can be seen that magnetite, ilmenite and olivine show an enrichment of bismuth, while pyroxene and plagioclase carry lesser amounts. The distribution of bismuth in the oxide minerals ilmenite and magnetite and the silicate minerals, plagioclase and pyroxene is put in perspective if one compares the ratios of the bismuth contents in these minerals (Table 4).

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TABLE 4. Distribution Coefficients for Bismuth in Coexisting Minerals

ROCK	*Plagioclase / Pyroxene*	*Ilmenite / Magnetite*	*Ilmenite / Plagioclase*	*Ilmenite / Pyroxene*	*Magnetite / Plagioclase*	*Magnetite / Pyroxene*
5181	1.1	1.3	3.0	3.3	2.2	2.5
4427	1.1	1.0	6.2	6.9	6.1	6.8
5052	1.1	0.8	4.6	5.4	6.0	7.0
5112	1.3	—	—	—	—	—
5092	0.9	—	—	—	—	—
AVERAGE	1.1	1.0	4.6	5.2	4.7	4.5

Two features are immediately apparent.

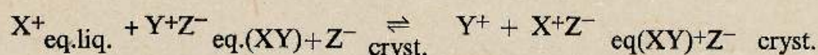
(*a*) The remarkable constancy of the distribution coefficient for the oxide mineral pair, ilmenite and magnetite and also for the silicate mineral pair plagioclase and pyroxene, their average values being 1.0 and 1.1, respectively.

(*b*) The distribution coefficient of approximately 5 for oxide/silicate pairs as shown by the last column of Table 4.

At this point of the discussion, one can safely conclude that bismuth shows a distinct preference for the oxide minerals as against the calcium bearing silicate minerals. Olivine has not been considered in a similar manner, due to the fact that only one set of data is available where olivine and the oxide minerals coexist. However, judging by the bismuth contents of the olivines analysed, it appears that olivine incorporates less bismuth than the oxide minerals.

The uptake of bismuth from the liquid into the available sites in the structures of the minerals studied can best be discussed if one considers the equilibrium constants of partition K.

The replacement of a cation Y^+ in a mineral structure by a trace element cation X^+ is governed by the equilibrium,



$$X^+_{eq.liq.} + Y^+Z^-_{eq.(XY)+Z^-\ cryst.} \rightleftharpoons Y^+ + X^+Z^-_{eq(XY)^+Z^-\ cryst.}$$

where each component is considered to be in the actual environment afforded by the liquid or solid of equilibrium composition.[23] The partition coefficient K of bismuth with respect to another element Y has been calculated for the different minerals, K being defined as

$$K = \frac{Bi^{3+}\ mineral}{Bi^{3+}\ liquid} \times \frac{Y^+\ liquid}{Y^+\ mineral}$$

where $Y^+ = Ca^{2+}$, Fe^{2+}, Fe^{3+}, Mg^{2+} and Ti^{4+} depending on the presence of these ions as major element cations in the structure of the minerals concerned. It is to be noted however, that in this work the activities have been replaced by concentrations.

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Table 5 shows the calculated partition coefficients of bismuth against the major cations in the minerals studied. The partition coefficient K of bismuth with respect to Ti^{4+} in ilmenite, shows the lowest value, while that for Fe^{2+} in magnetite shows the highest. However, except for a single K value of 1.02 for Fe^{2+} in magnetite, all other K values in Table 5 are less than unity. This indicates the fact that bismuth prefers to remain in the liquid as against the mineral phases. A better idea of the relative partition coefficients of bismuth with respect to the major element cations could be obtained if the K values are recalculated taking the average K value for Ti^{4+} (which is the least) as unity (Table 5). When the other K values are divided by the average K Ti^{4+} one arrives at the following decreasing order for the partition coefficients.

(1) Magnetite Fe^{2+}
(2) Ilmenite Fe^{2+}
(3) Pyroxene Fe^{2+}
(4) Plagioclase Ca^{2+}
(5) Olivine Fe^{2+}
(6) Pyroxene Ca^{2+}
(7) Olivine Mg^{2+}
(8) Pyroxene Mg^{2+}
(9) Magnetite Fe^{3+}
(10) Ilmenite Ti^{4+}

TABLE 5. Equilibrium Constants for Partition (K)

ROCK	*Il* Ti^{4+}	*Mag* Fe^{3+}	*Pyrox* Mg^{2+}	*Ol* Mg^{2+}	*Pyrox* Ca^{2+}	*Ol* Fe^{2+}	*Plag* Ca^{2+}	*Pyrox* Fe^{2+}	*Il* Fe^{2+}	*Mag* Fe^{2+}
5181	0.07	0.07	0.07	0.16	0.24	0.41	0.55	0.45	0.61	0.70
4427	0.07	0.07	0.06	—	0.10	—	0.21	0.21	0.43	0.64
5052	0.09	0.12	0.12	—	0.18	—	0.33	0.36	0.52	1.02
5112	—	—	0.20	0.10	0.30	0.30	0.65	0.67	—	—
5092	—	—	0.19	0.16	0.18	0.47	0.36	0.58	—	—
Average	0.07	0.08	0.12	0.14	0.20	0.39	0.42	0.45	0.52	0.78
Relative	1.00	1.14	1.71	2.00	2.85	5.57	6.00	6.42	7.42	11.14

K values taking K for Ti^{4+} in ilmenite as unity.

From these decreasing order of K values, a few salient features emerge which are worthy of note.

(*a*) The relatively high placings for the Fe^{2+} sites in minerals.

(*b*) The very low placing for the triple and quadruple charged Fe^{3+} and Ti^{4+}.

(*c*) The higher placing for Fe^{2+} sites as against the Mg^{2+} sites for both pyroxene and olivine.

(*d*) The higher placing for the plagioclase Ca^{2+} as against the pyroxene Ca^{2+}.

From Table 6 where the ratios of the partition coefficients are shown, it is at once apparent how much in the oxide minerals (which are the best hosts for Bi), bismuth shows greater preference for the Fe^{2+} sites as against the Fe^{3+} and Ti^{4+} sites.

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TABLE 6. Partition Coefficient Ratios for the Different Cations

ROCK	$\frac{Mag\,Fe^{2+}}{Mag\,Fe^{3+}}$	$\frac{Il.Fe^{2+}}{Il.Ti^{4+}}$	$\frac{Pyrox.Fe^{2+}}{Pyrox.Mg^{2+}}$	$\frac{Ol.Fe^{2+}}{Ol.Mg^{2+}}$	$\frac{Mag.Fe^{2+}}{Pyrox.Fe^{2+}}$	$\frac{Pyrox.FeCa^{2+}}{Pyrox.Ca^{2+}}$	$\frac{Plag.Ca^{2+}}{Pyrox.Ca^{2+}}$	$\frac{Pyrox.Mg^{2+}}{Ol.Mg^{2+}}$	$\frac{Mag.Fe}{Il.Fe^{2}}$	$\frac{Pyrox.Fe^{2+}}{Ol.Fe^{2+}}$
5181	10.00	8.71	6.42	2.56	1.55	1.87	2.29	0.43	1.14	1.09
4427	9.14	6.14	3.50	—	3.04	2.10	2.10	—	1.48	—
5052	8.50	5.77	3.00	—	2.83	2.00	1.83	—	1.96	—
5112	—	—	3.35	3.00	—	2.23	2.16	2.00	—	2.23
5092	—	—	3.05	2.93	—	3.22	2.00	1.18	—	1.23
Average	9.21	6.87	3.86	2.83	2.47	2.28	2.07	1.53	1.52	1.51

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4. Conclusions

From the above discussion the following conclusions could be arrived at:

(1) A smooth variation of the geochemical behaviour exists as the atomic weight increases from As to Bi.

(2) The geochemical behaviour of arsenic is characterized by the predominance of the As^{5+} ion and the dual ability to act as a network former and a network modifier.

(3) In the minerals, arsenic substitutes for Al^{3+}, Fe^{3+}, Ti^{4+} and Si^{4+}.

(4) Antimony also contains both the tri- and pentavalent ions in the minerals even though the Sb^{3+} ion predominates over the Sb^{5+} ion.

(5) The stability of the trivalent ion is highest in bismuth, where it shows preferential entry into the oxide minerals as against the silicate minerals. Within the oxide minerals Bi prefers the Fe^{2+} sites to Fe^{3+} and Ti^{4+} sites.

The group V elements provide a very good example of how the geochemical distribution follows the trends expected from their electronic configuration, ionic size and general chemical properties.

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Decarbonation Reactions and the Origin of Vein-Graphite in Sri Lanka

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(*Paper accepted*: 15 *October* 1976)

Abstract: The central question in this problem of the origin of vein-graphite is the source of the carbon. This question can only be viewed against the metamorphic background of the rocks of the island. The CO_2 derived from decarbonation reactions in the calcareous environment provided an adequate source of the required carbon and is postulated to have entered into rock-structures such as joint-planes, etc., and reacted with the country rocks to produce carbon. The practical significance of this theory is that we can now recognise the geological environment in which vein-graphite can occur.

1. Introduction

The two main modes of occurrence of graphite in Sri Lanka are (1) as disseminated flakes in the country rocks such as granulites, charnockites, crystalline limestones, graphite schists, etc. forming the Highland Series, and (2) as veins occupying fractures in the rock. The disseminated flakes evidently represent metamorphosed organic carbonaceous matter in the original rocks. Vein-graphite is not of the same origin because it is not an original rock-forming mineral as the disseminated graphite but occurs in the rock structures such as joints and faults, mostly joints, oblique, transverse or longitudinal in orientation and is therefore of later origin.

It will be of interest to compare the C12/C13 ratios for vein-graphite of inorganic origin and for disseminated graphite in the country rocks (charnockite-metasedimentary series) evidently of organic origin.

Some of the earlier views regarding the origin of graphite are contained in a paper by Wadia[9] and more recently by Erdosh.[3] Wadia postulated the theory of absorption of limestone by charnockitic intrusions resulting in the production of various lime-silicates and pyroxenes in the magma and the elimination of carbon in gaseous or volatile form. Wadia, in keeping with the geological thinking of the times, regarded all charnockitic rocks as being intrusive into what was then known as rocks of the Khondalite system. Regional geological mapping carried out by the Geological Survey Department during the last 20 years has shown that the charnokites, with the exception of a few minor local occurrences, bear no intrusive relationships to the quartites, granulites and marbles of the Khondalite Series and occur interbanded and conformable with these rocks to form what was at first known as the charnockite-metasedimentary series and subsequently termed the Highland Series.

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Charnockites have thus been shown to form an inseparable part of one and the same succession. Therefore, the very premise from which Wadia attempted to build up his theory is shown to be erroneous and his views cannot therefore be taken into consideration to explain the origin of vein-graphite.

2. Decarbonation reactions

The central question in this problem of the origin of vein-graphite is the source of the carbon, a question which can only be dealt with in a background of elucidation of the metamorphic processes to which the rocks of the island have been subjected. These processes are marked by metamorphic reactions in different petro-chemical environments such as pelitic, calcareous and basic, only the decarbonation reactions in the calcareous environment bearing relevance to this question.[4,5,6,7,1,8]

In addition to calcite and dolomite, the crystalline limestones (marbles) of the Highland Series consist of a variety of minerals such as forsterite, diopside, phlogopite, spinel, apatite and graphite and some of the mineral assemblages recognised are as follows :—

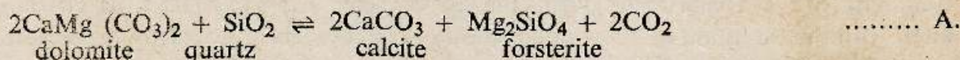
1. Calcite ± dolomite — forsterite.
2. Calcite ± dolomite — forsterite — diopside.
3. Calcite ± dolomite — forsterite — phlogopite.
4. Calcite ± dolomite — forsterite — diopside — phlogopite.

Spinel, apatite or graphite may be additional minerals in any of these assemblages.

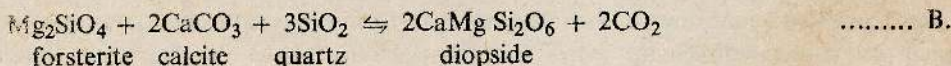
The calc-granulites and gneisses consist of different combinations of minerals such as diopside, scapolite, calcite, wollastonite, sphene and perthite. The relevant assemblages are only those which bear wollastonite and these assemblages are as follows :—

1. Diopside — scapolite — wollastonite ± sphene.
2. Diopside — scapolite — wollastonite — perthite ± sphene.

Considering both the 1st and 2nd periods of granulite facies metamorphism to which the rocks of the island have been subjected, the type of decarbonation reactions involved in the production of the above assemblages are as follows :—



$$\underset{\text{dolomite}}{2CaMg(CO_3)_2} + \underset{\text{quartz}}{SiO_2} \rightleftharpoons \underset{\text{calcite}}{2CaCO_3} + \underset{\text{forsterite}}{Mg_2SiO_4} + 2CO_2 \quad \text{......... A.}$$



$$\underset{\text{forsterite}}{Mg_2SiO_4} + \underset{\text{calcite}}{2CaCO_3} + \underset{\text{quartz}}{3SiO_2} \leftrightharpoons \underset{\text{diopside}}{2CaMgSi_2O_6} + 2CO_2 \quad \text{......... B.}$$

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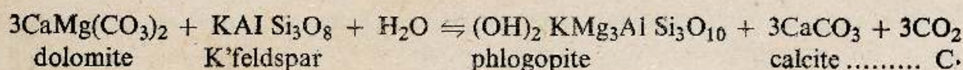

$$3CaMg(CO_3)_2 + KAlSi_3O_8 + H_2O \rightleftharpoons (OH)_2KMg_3AlSi_3O_{10} + 3CaCO_3 + 3CO_2$$
dolomite K'feldspar phlogopite calcite .... C.

$$CaCO_3 + SiO_2 \rightleftharpoons CaSiO_3 + CO_2$$
calcite quartz wollastonite .... D.

3. Discussion of the CO_2 — producing reactions and source of the carbon.

The complete elimination of dolomite and quartz in reaction A resulted in calcite-forsterite with release of CO_2. The elimination of quartz in A resulted in calcite-dolomite-forsterite with release of CO_2. The elimination of quartz in reaction B resulted in the assemblage calcite-forsterite-diopside with CO_2 released. The elimination of dolomite in reaction A resulted in the association calcite-forsterite-quartz with release of CO_2. This assemblage is unstable in the pyroxene granulite subfacies, forsterite reacting with calcite in the presence of quartz to yield diopside and CO_2 according to reaction B ; this reaction proceeded until quartz was completely eliminated resulting in the assemblage calcite-forsterite-diopside with CO_2 released. Thus, calcite-forsterite-quartz apparently stable in the garnet-diopside-quartz subfacies was converted to calcite-forsterite-diopside (with CO_2 released) through reaction B under conditions of the pyroxene-granulite subfacies. Any potash feldspar present in forsterite-bearing marbles (pyroxene-granulite subfacies) would have reacted with dolomite in the presence of water to yield phlogopite with release of CO_2 according to reaction C under conditions of the hornblende-granulite subfacies. This reaction ceased when all the potash feldspar was consumed resulting in the association calcite-dolomite-forsterite-phlogopite. The elimination of calcite and quartz in reaction D resulted in the production of wollastonite with CO_2 released.

From the above mentioned reactions it is important to note : (1) that the CO_2 released provided an entirely adequate source of the carbon required for the production of vein-graphite, (2) that these reactions produced minerals such as forsterite, diopside, phlogopite and also wollastonite, the significance of which is explained below.

The CO_2 derived from the reactions found its way into joint-planes, fault-planes, etc. and reacted with the country rock with which it came into contact to produce carbon by means of some reaction, at present not known ; however, the type of carbon-producing reaction suggested, is as follows :

$CO_2 + 4FeO = 2Fe_2O_3 + C$.[3] Such a mechanism is postulated to explain the origin of vein-graphite.

Impure marbles bearing forsterite, forsterite-diopside, forsterite-phlogopite or forsterite-diopside-phlogopite and wollastonite-bearing calc-granulities are most significant because they have been shown to be the source of the CO_2 which in turn provided the source of the carbon required for the formation of vein-graphite.

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That is, a genetic relationship is clearly seen between calcareous rocks such as forsterite, forsterite-diopside, forsterite-phlogopite or forsterite-diopside-phlogopite bearing marbles and wollastonite-bearing calc-granulites on the one hand and vein-graphite on the other, thus implying that the environment in which vein-graphite occurs is marked by the presence of such calcareous rocks, although such rocks may not always be seen in the vicinity of graphite veins, in which case, they may well be found to occur at depth, as for example at the Bogala Mines at Kotiyakkumbara and also, as recent investigations showed, at Katuwana, near Morawaka. Wadia[9] too, had observed a close association of calcareous strata with graphite bodies.

4. Practical significance

The geological environment in which vein-graphite can occur must satisfy either or both of the following requirements : (1) the environment is marked by the presence of impure marbles bearing minerals such as forsterite, forsterite-diopside, forsterite-phlogopite or forsterite-diopside-phlogopite, especially when these minerals are present in larger amounts. It is noted that pure marbles or impure marbles bearing minerals other than those above mentioned (i.e. spinel, apatite or sphene) are of no significance from the point of view of vein-graphite; (2) the environment is marked by the presence of wollastonite-bearing calc-granulites.

The practical significance of this theory is that we can now recognise the geological environment, as defined above, in which vein-graphite can occur and that any search for commercial graphite bodies either on the surface or in depth should be confined to this particular environment, especially when marbles show an abundance of the above mentioned minerals.

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Two Diophantine Equations in Cyclotomic Fields

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(*Paper accepted: 6 January* 1977)

Abstract : If p, g are distinct odd rational primes, it is shown that the diophantine equations $|\alpha|^2 = p^2$ and $|\alpha|^2 = p$ have no solutions in integers belonging to the cyclotomic field $K = R(e^{2\pi i/g})$, if $g > \frac{1}{2}p^{p2/3}$. It is also shown that there are values of p and g, other than those satisfying $g = p$ or $g = p^2 + p + 1$, for which the two equations have non-trivial solutions in integers belonging to K.

1. Introduction

Let p and g be distinct odd rational primes. Ankeny and Chowla[1] have proved that the equations

$$|\alpha|^2 = p^2 \quad (1)$$

and

$$|\alpha|^2 = p \quad (2)$$

have no non-trivial solutions in integers α belonging to the cyclotomic field $K = R(e^{2\pi i/g})$, if $g > p^{p2}$. In section 2, we show that the condition $g > p^{p2}$ can be replaced by $g > \frac{1}{2}p^{p\frac{2}{3}}$ while in section 3 we obtain values of p and g for which the equations (1) and (2) have non-trivial solutions in integers belonging to K.

2. Condition for non-solvability

Theorem 1. If $g > \frac{1}{2}p^{p\frac{2}{3}}$, the equation (1) has no solutions in integers α belonging to the field K apart from the trivial ones, namely, $\alpha = \pm p\theta^r$, $\alpha = \pm p$, where r is prime to g, and $\theta = e^{2\pi i/g}$.

Proof. Write

$$\alpha = T(\theta) = c_0 + c_1\theta + c_2\theta^2 + \dots + c_{g-1}\theta^{g-1}$$

where $c_0, c_1, c_2, \dots, c_{g-1}$ are defined.[1]

Then, if α is a solution of equation (1), it can be shown,[1] that

$$T(\theta) = c_0 + c_1(\theta + \theta^p + \theta^{p2} + \dots + \theta^{p^{f-1}}) + c_2(\theta^i + \theta^{ip} + \theta^{ip2} + \dots + \theta^{ip^{f-1}} + c_j(\theta^j + \theta^{jp} + \theta^{jp2} + \dots + \theta^{jp^{f-1}}) + \dots\dots$$

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where f is the least positive integer such that

$$p^f \equiv 1 \pmod{g} \tag{3}$$

and $i \equiv p^a$, $j \equiv p^b$, $(j|i) \equiv p^d \pmod{g}$, etc.,

and that

$$c_0^2 + f\,(c_1^2 + c_i^2 + c_j^2 + \ldots\ldots) = p^2 \tag{4}$$

and

$$c_0 + f\,(c_1 + c_i + c_j + \ldots\ldots) = \pm p \tag{5}$$

Consider the following three cases: –

Case (*i*). Suppose that $c_0 \neq 0$ and that only one of $c_1, c_i, c_j, \ldots\ldots$, say c_t, is non-zero.

Then, equations (4) and (5) reduce to

$$c_0^2 + f\,c_t^2 = p^2$$

and

$$c_0 + f\,c_t = \pm p.$$

But, since $c_t \neq 0$ and $f \neq 0$, the above two equations give

$$c_0 = \frac{1-f}{2}\,c_t$$

and so $c_0 = \pm\dfrac{1-f}{2}$ and $c_t = \pm 1$, as $(c_0, c_t) = 1$.

Whence

$$p^2 = \left(\frac{1-f}{2}\right)^2 + f = \left(\frac{1+f}{2}\right)^2$$

and therefore

$$p = \frac{1+f}{2} > \frac{f}{2}. \tag{6}$$

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By equation (3), $p^f = 1 + \lambda g$, where λ is an even positive integer.

Hence,

$$f \log p = \log (1 + \lambda g) > \log 2g,$$

and so

$$f > \frac{\log 2g}{\log p}. \tag{7}$$

From equations (6) and (7), we obtain

$$2p > \frac{\log 2g}{\log p},$$

which gives

$$g < \tfrac{1}{2} p^{2p} < \tfrac{1}{2} p^{p^{3/2}}, \quad \text{if } p \geqslant 7.$$

Take the exceptional cases $p = 3$ and $p = 5$.

When $p = 3$, we have from equation (6), $f = 5$, and therefore equation (3) gives

$$3^5 \equiv 1 \pmod{g},$$

which implies that the only possible value of g is

$$g = 11 < \tfrac{1}{2} p^{p^{3/2}}.$$

When $p = 5$, we have from equation (6), $f = 9$ and equation (3) gives

$$5^9 \equiv 1 \pmod{g},$$

which implies that the only possible values of g are $g = 19, 31$ or 829, and for each of these values of g,

$$g < \tfrac{1}{2} p^{p^{3/2}}.$$

Thus, in this case, equation (1) has no non-trivial solutions if

$$g > \tfrac{1}{2} p^{p^{3/2}}.$$

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Case (ii). Suppose only two of c_1, c_i, c_j,......say c_t and c_u, are non-zero.

First, suppose $c_t = \pm 1$ and $c_u = \pm 1$.

Then equations (4) and (5) reduce to

$$c_0^2 + 2f = p^2 \tag{8}$$

and

$$c_0 + f(\pm 1 \pm 1) = \pm p. \tag{9}$$

But, since $c_0 \neq \pm p$ as $f \neq 0$, it follows that c_t and c_u must take the same sign and so equation (9) gives

$$c_0 \pm 2f = \pm p. \tag{10}$$

Since $f \neq 0$, from equations (8) and (10), we get $2(f \pm c_0) = 1$, which is clearly impossible. Hence, our supposition that $c_t = \pm 1$ and $c_u = \pm 1$ is false, and so it easily follows from equation (4) that $p^2 \geqslant 5f$.
Proceeding as in case (i), it can be shown that

$$g < \tfrac{1}{2} p^{p^{\frac{5}{2}}} < \tfrac{1}{2} p^{p^{\frac{2}{3}}}.$$

Thus in this case, equation (1) has no non-trivial solution if

$$g > \tfrac{1}{2} p^{p^{\frac{2}{3}}}.$$

Case (iii). Suppose $n\ (\geqslant 3)$ of c_1, c_i, c_j,......are non-zero.

Then, from equation (4) we have

$$p \geqslant n f$$

Proceeding as in case (i), it can be shown that

$$g < \tfrac{1}{2} p^{p^{\frac{2}{n}}} \leqslant \tfrac{1}{2} p^{p^{\frac{2}{3}}}, \quad \text{since } n \geqslant 3.$$

Thus, in this case also equation (1) has no non-trivial solutions if

$$g > \tfrac{1}{2} p^{p^{\frac{2}{3}}}.$$

The proof of the theorem is now complete.

The next theorem follows directly from Theorem 1.

Theorem 2. If $g > \frac{1}{2} p^{p^{\frac{2}{3}}}$, the equation (2) is impossible in integers α belonging to the field K.

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3. Equations with non-trivial solutions

Theorem 3. If p and g are odd primes such that p is a primitive root of g, then the equation (1) has no non-trivial solutions in integers α belonging to the field K.

Proof. Since p is a primitive root of g, we have $f = g - 1$ and so

$$c_1 = c_2 = c_3 = \ldots\ldots = c_{g-1}.$$

Hence,

$$\alpha = T(\theta) = c_0 + c_1 (\theta + \theta^2 + \theta^3 + \ldots\ldots + \theta^{g-1})$$
$$= c_0 - c_1$$

and we obtain a trivial solution.

The theorem now follows.

Corollary. Under the conditions of Theorem 3, the equation (2) is impossible in integers α belonging to the field K.

Theorem 4. Let p and g be odd primes such that $g = 2p + 1$, $p \equiv 1 \pmod 4$ and $2p - 1 = k^2$, where k is a rational integer.

Then

$$\alpha = \frac{k+1}{2} + \theta^2 + \theta^{2p} + \theta^{2p^2} + \ldots\ldots + \theta^{2p^{p-1}}$$

is a solution of the equation (2).

Proof. Since $(2|g) = -1$, as $g = 2p + 1$ and $p \equiv 1 \pmod 4$, 2 is a quadratic non-residue of g and so by Euler's Criterion,

$$2^{\frac{g-1}{2}} \equiv -1 \pmod g.$$

Whence,

$$2^p \equiv -1 \pmod g.$$

Since $g > 3$, it follows that $2^t \not\equiv -1 \pmod g$ for $1 \leqslant t \leqslant p$. But, since $2p \equiv -1 \pmod g$, we have

$$(2p)^p = -1 \pmod g,$$

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and so $p^p \equiv 1 \pmod{g}$ and $p^t \not\equiv 1 \pmod{g}$ for $1 \leqslant t < p$.

Also $(p|g) = 1$.

Hence, $2, 2p, 2p^2, \ldots\ldots, 2p^{p-1}$ are incongruent quadratic non-residues modulo g. But, all the $\frac{g-1}{2}$ incongruent quadratic non-residues modulo g are given by $-1^2, -2^2, -3^2, \ldots\ldots, -\left(\frac{g-1}{2}\right)^2$ and so $2, 2p, 2p^2, \ldots\ldots, 2p^{p-1}$ are congruent to $-1^2, -2^2, -3^2, \ldots\ldots, -\left(\frac{g-1}{2}\right)^2$ modulo g, in some order.

Hence,

$$\alpha = \frac{k+1}{2} + \theta^2 + \theta^{2p} + \theta^{2p^2} + \ldots\ldots + \theta^{2p^{p-1}}$$

$$= \frac{k+1}{2} + \theta^{-1^2} + \theta^{-2^2} + \theta^{-3^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2}$$

and therefore

$$\alpha = \frac{k+1}{2} + \theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{-\left(\frac{g-1}{2}\right)^2}.$$

Since $1^2, 2^2, 3^2, \ldots\ldots, \left(\frac{g-1}{2}\right)^2$ are the incongruent quadratic residues modulo g, and $\left(\frac{g+1}{2}\right)^2, \left(\frac{g+3}{2}\right)^2, \ldots\ldots, (g-1)^2$ are also $\frac{g-1}{2}$ incongruent quadratic residues modulo g, these must be congruent to $1^2, 2^2, 3^2, \ldots\ldots\left(\frac{g-1}{2}\right)^2$ modulo g, in some order. Hence, the Gaussian sum

$$\phi(1, g) = 1 + \theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{(g-1)^2}$$

$$= 1 + 2\left(\theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2}\right)$$

But, $\phi(1, g) = i\sqrt{g}$, since $g \equiv 3 \pmod{4}$.

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Therefore,

$$1 + 2 \left(\theta^{1^2} + \theta^{2^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2} \right) = i\sqrt{g},$$

which gives

$$\theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2} = \frac{-1 + i\sqrt{g}}{2}.$$

Hence,

$$\alpha = \frac{k+1}{2} + \frac{-1 + i\sqrt{g}}{2} = \frac{k}{2} + i\,\frac{\sqrt{g}}{2}$$

and

$$\bar{\alpha} = \frac{k}{2} - i\,\frac{\sqrt{g}}{2}$$

therefore

$$|\alpha|^2 = \frac{k^2 + g}{4} = p.$$

The theorem now follows.

Theorem 5. If p and g are odd primes such that $g = 4p - 1$ and the order of p modulo g is $2p - 1$, then

$$\alpha = 1 + \theta^2 + \theta^{2p} + \theta^{2p^2} + \ldots\ldots + \theta^{2p^{(2p-2)}}$$

is a solution of the equation (2).

Proof. Since the order of p modulo g is $2p - 1$, it follows that $2, 2p, 2p^2, \ldots\ldots\ldots\ldots, 2p^{2p-2}$ are incongruent modulo g.

Since $g = 4p - 1$, we have $(2|g) = -1$ and $(p|g) = 1$.

Thus, $(2p^r|g) = -1$, for any integer r, such that $0 \leqslant r \leqslant 2p - 2$.

Hence, $2, 2p, 2p^2 \ldots\ldots, 2p^{2p-2}$ are incongruent quadratic non-residues modulo g.

As in the proof of Theorem 4, it can be shown that these must be congruent to $-1^2, -2^2, -3^2, \ldots\ldots, -\left(\frac{g-1}{2}\right)^2$ modulo g, in some order, and so,

$$\begin{aligned} \alpha &= 1 + \theta^2 + \theta^{2p} + \theta^{2p^2} + \ldots\ldots + \theta^{2p^{2p-2}} \\ &= 1 + \theta^{-1^2} + \theta^{-2^2} + \theta^{-3^2} + \ldots\ldots + \theta^{-\left(\frac{g-1}{2}\right)^2}. \end{aligned}$$

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Therefore,

$$\bar{\alpha} = 1 + \theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2}.$$

Now the Gaussian sum,

$$\phi(1, g) = 1 + \theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{(g-1)^2}$$

$$= 1 + 2\left(\theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2}\right).$$

$$= i\sqrt{g}, \text{ since } g \equiv 3 \pmod 4).$$

Hence,

$$\theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2} = \frac{-1 + i\sqrt{g}}{2}$$

and therefore

$$\bar{\alpha} = \frac{1 + i\sqrt{g}}{2} \text{ and } \alpha = \frac{1 - i\sqrt{g}}{2}$$

Whence,

$$|\alpha|^2 = \frac{1 + g}{4} = p.$$

The next two theorems can be easily verified using the Gaussian sum.

Theorem 6. If p and g are odd primes such that $4p = a^2 + b^2 g$, where a and b are coprime odd integers, then the equation (2) has a non-trivial solution in K, given by:

$$\alpha = \frac{a - b}{2} + b\left(1 + \theta^{1^2} + \theta^{2^2} + \ldots\ldots + \theta^{\left(\frac{g-1}{2}\right)^2}\right)$$

Theorem 7. If p and g are odd primes such that $p = a^2 + b^2 g$, $g \equiv 3 \pmod 4$, where a, b are coprimes rational integers of opposite parity, then the equation (2) has a non-trivial solution in the field K given by

$$\alpha = a + b\left(1 + \theta^{1^2} + \theta^{2^2} + \theta^{3^2} + \ldots\ldots + \theta^{(g-1)^2}\right.$$

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An Infinite Optical Path Photoreactor and a Filter for the Isolation of Light at 366 NM

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Abstract : It is well known that the conventional optical bench photoreactors have many shortcomings such as inefficient utilisation of light, calibration of absorbed light intensity, etc. A new reactor, namely, the infinite optical path photoreactor, which eliminates most of the shortcomings is discussed. A theory for this reactor is derived. A new chemical filter for the isolation of light at 366 nm is shown to be effective.

1. Introduction

A photochemical reaction system differs from a normal chemical system in that the former has an additional feature which allows the exposure of the reaction mixture to a monochromatic light of definite intensity. Therefore, the design of a photochemical reaction system requires special attention as to

(i) the proper choice of construction material and filter,

(ii) the proper positioning of the light source,

(iii) the thermostating of the reaction vessel to prevent the temperature of the reaction system rising due to continued irradiation,

(iv) precautions for the prevention of loss of light due to reflection,

(v) the maximum light utilization efficiency.

The conventional (optical bench) photoreactor suffers from the following drawbacks :

(i) Inefficient utilization of light due to collimation difficulties.

(ii) Calibration of the absorbed light intensity is not possible as it is a function of the reactants.

(iii) Correction for the reflection and scattering of light at the glass-reactants interface is necessary.

(iv) Gradual variation of intensity of light absorbed within the reaction mixture, due to the consumption of the reactants, may cause changes in mechanism.

(v) Gradual variation of intensity with distance may cause different mechanisms to operate at different points in the solution.

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An 'infinite optical path photoreactor' (Figure 1) was designed which partially removed the above said defects. The unique feature of the reactor is its 100% light absorption and as a consequence of this, the following advantages are accomplished :

(i) Increased power utilization efficiency.

(ii) Easy calibration by only one actinometry experiment.

(iii) There is no variation of absorbed intensity (at any point in the reaction mixture) with time, because emitted light is totally absorbed by the reaction mixture, whatever its concentration, due to the infinite optical path available for absorption.

(iv) The variation of absorbed intensity with position is less marked (than in an optical bench photoreactor) due to the averaging effect caused by the periodic retracing of the path of a given ray due to multiple reflections.

(v) The correction for reflection at interface is irrelevant in this case due to the fact that there is no provision for light loss due to the geometry of the reactor.

2. Theoretical

The most spectacular feature of the reactor is its infinite optical path. The condition under which this photoreactor is of infinite optical path will be derived below.

Let the source be designated by 0 and the first reflection at the wall by 1, the second reflection at the wall by 2 and the third reflection by 3, etc.

Let α, β and γ be the optical densities of the filter system, reaction mixture and the pyrex wall of the reaction vessel, respectively, where

$$\alpha = \varepsilon_f C_f l_f + \varepsilon_{CA} C_{CA} l_{CA} + \varepsilon_{MB} C_{MB} l_{MB}$$

as the filter system (in this case) is made of two concentric cylindrical compartments containing chrome alum (CA) and methylene-blue (MB) separately (Figure 2).

$$\beta = \varepsilon_r C_r l_r \text{ (for the reaction mixture)}$$

$$\gamma = \varepsilon_\omega C_\omega l_\omega$$

where f = filter (pyrex) glass

r = reaction mixture

ω = pyrex glass wall

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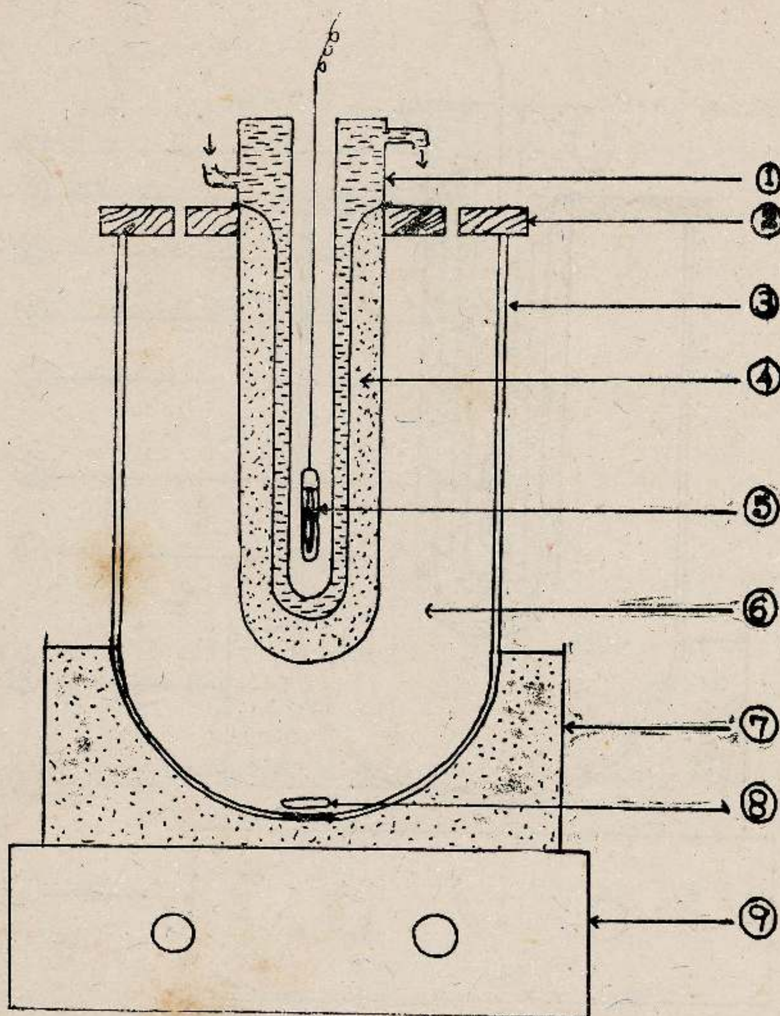

Figure 1. Infinite optical path photoreactor

1. Water jacket
2. Wooden lid
3. Thermos flask
4. Filtor solution (CA)
5. Mercury lamp (medium pressure)
6. Reaction mixture
7. Regiform box
8. Magnetic needle
9. Magnetic stirrer

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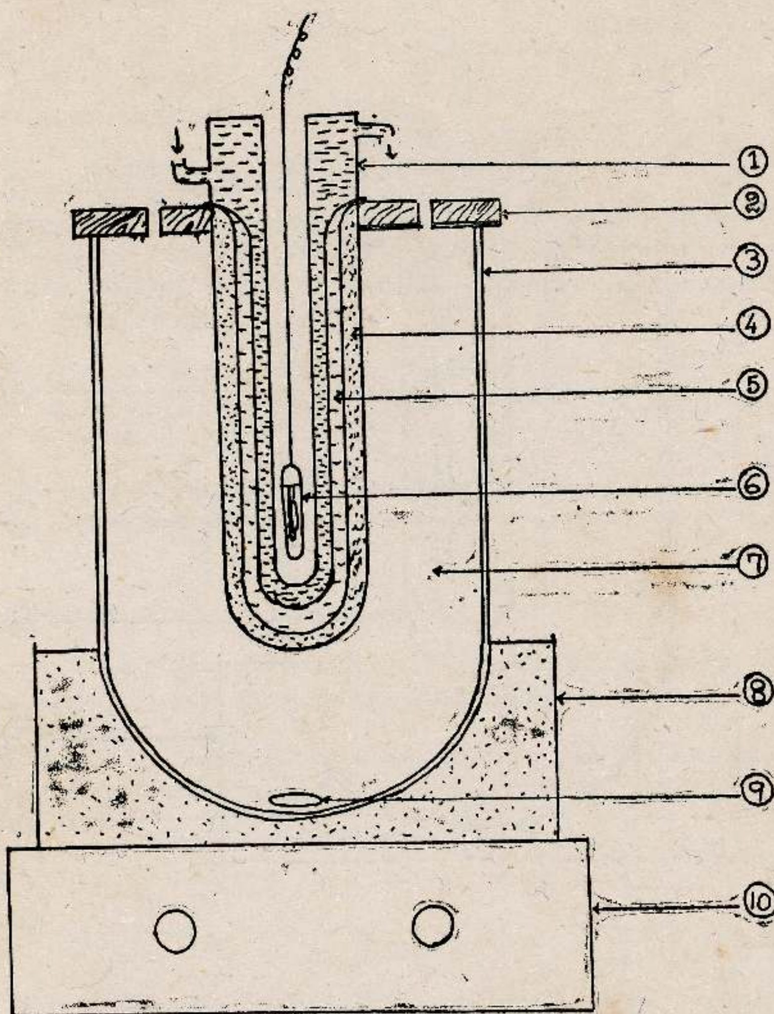

Figure 2. Infinite optical path photoreactor

1. Water jacket
2. Wooden lid
3. Thermos flask
4. Filter solution (MB)
5. Filter solution (CA)
6. Mercury lamp (medium pressure)
7. Reaction mixture
8. Regiform box
9. Magnetic needle
10. Magnetic stirrer

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Let I_{ij} be the intensity of light absorbed by the reaction mixture when the light travels from i to j where

$i = 0, 1, 2......$ and $j = i + 1$ (i.e. $i \in N$)

Assuming that there is no reflection at the interfaces and losses through the opening (Figure 2),

by Beer-Lambert's law,

$I_{oi} = I_o(\lambda)\ 10^{-\alpha}\ (1 - 10^{-\beta})$ where

$I_o(\lambda)$ is the intensity of light emitted at λ *nm*

$$I_{12} = I_o(\lambda)\, 10^{-(\alpha + \beta)}\, 10^{-\gamma}\, (1 - 10^{-\beta}) + I_o(\lambda)\, 10^{(3\alpha + \beta + \gamma)}\, (1 - 10^{-\beta})$$

$$I_{23} = I_o(\lambda)\, 10^{-(3\alpha + 2\beta + \gamma)}\, (1 - 10^{-\beta}) + I_o(\lambda)\, 10^{-(5\alpha + 3\beta + 2\gamma)}\, (1 - 10^{-\beta})$$

$$I_{34} = I_o(\lambda)\, 10^{-(5\alpha + 4\beta + 3\gamma)}\, (1 - 10^{-\beta}) + I_o(\lambda)\, 10^{-(7\alpha + 5\beta + 2\gamma)}\, (1 - 10^{-\beta})$$

Total intensity of light absorbed by the reaction mixture (I_{abs}) is given by the following equation

$$I_{abs} = \sum_{i=0} I_{ij} \quad (j = i + 1)$$

$$I_{abs} = I_o(\lambda)\, 10^{-\alpha}\, (1 - 10^{-\beta}) \left\{ 1 + \frac{1}{10^{(\beta + \gamma)}} + \frac{1}{10^{(2\alpha + \beta + \gamma)}} \cdots\cdots\cdots + \frac{1}{10^{(2\alpha + 2\beta + 2\gamma)}} + \frac{1}{10^{(4\alpha + 3\beta + 2\gamma)}} \right\} + \cdots\cdots\cdots$$

It can be easily seen that $I_{abs} = I_o(\lambda)$ only when $\alpha = 0$ and β and/or γ are very large. For 100% absorption of light by the reactants, the wall should not absorb any light but should reflect it. Therefore $\lambda = 0$ in order to satisfy the requirement.

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In short, α and γ should be zero and β should be very large for the 100% absorption by the reaction mixture and for infinite optical pathlength. In other words, the infinite optical path photoreactor functions with 100% efficiency when, and only when, the glass and filter solutions do not absorb any light and the mixture absorbs it strongly.

In order to make $\alpha = 0$, we must find a filter which possesses 100% transmission at the wavelength concerned. To make $\gamma = 0$, a noble metal surface can be used.

3. Chemical Filter

Chemical filters are often used with medium pressure mercury lamps to provide an intensity of monochromatic light which is often higher than those obtainable from conventional photochemical monochromator systems.

The main interest has been the mechanistic study of photochemical benzpinacolization. Benzophenone reduction in the presence of a hydrogen donor requires an irradiation of light of wavelength 366 nm, because, the energy gap between the first excited singlet of benzophenone ($^1S_1$) and ground state ($^1S_0$) matches the energy of light of wavelength 366 nm. For any quantitative and easily interpretative measurement, one should have a monochromatic light. Firstly, the construction of a near perfect monochromatic 'chemical filter' will be considered.

In the early stages of the investigation of the photoreduction of benzophenone, an aqueous solution of chrome alum had been used[4] as a chemical filter which transmits light in the region $450 < \lambda < 550$ nm within which is a Hg band at 546 nm (Figures 3 and 5). So the filter is not monochromatic and should therefore be modified. Attempts were made to improve both the monochromaticity and transmittance of the filter. A study of transmittance characteristics of various compounds was made. The selection of the filter compounds was based on the criterion that a violet aqueous solution was obtained with transmission in the region $350 < \lambda < 400$ nm. Some of them were aqueous solutions of crystal violet, chrome alum, gentian violet, bromothymol blue and methylene blue. None of the above mentioned solutions gave a transmittance peak having a maximum close to $\lambda = 366$ nm. However the transmission spectrum of methylene blue (Figure 4) is noteworthy. Although it transmits light at 400 nm and 435 nm, where there are Hg bands, (Figure 5), it does not transmit light at 546 nm. Further, this does not have transmission in the region $\lambda < 350$ nm and $\lambda > 500$ nm. A desirable feature of methylene blue is that one of its maxima is at $\lambda = 375$ nm which is very close to 366 nm. Therefore, it was expected that a mixture of CA and MB would yield a near perfect filter. Using the transmission versus wavelength plot (Figure 3) of chrome alum and assuming Beer-Lambert's law, the optimum concentration of chrome alum was calculated by maximising the transmission at 366 nm and minimising the transmission at 540 nm. The optimum concentration was estimated to be 0·28 moles l^{-1} (with 10% transmission at 310 nm and 366 nm, and 0·05% transmission at 540 nm).

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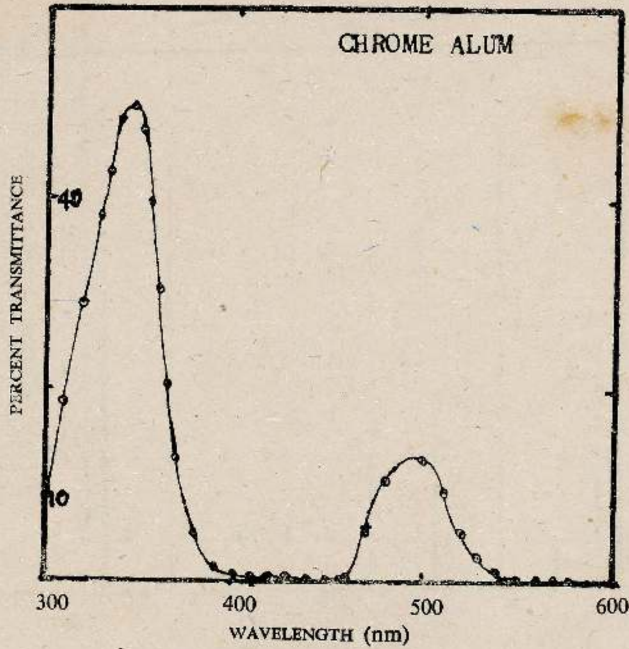

Figure 3

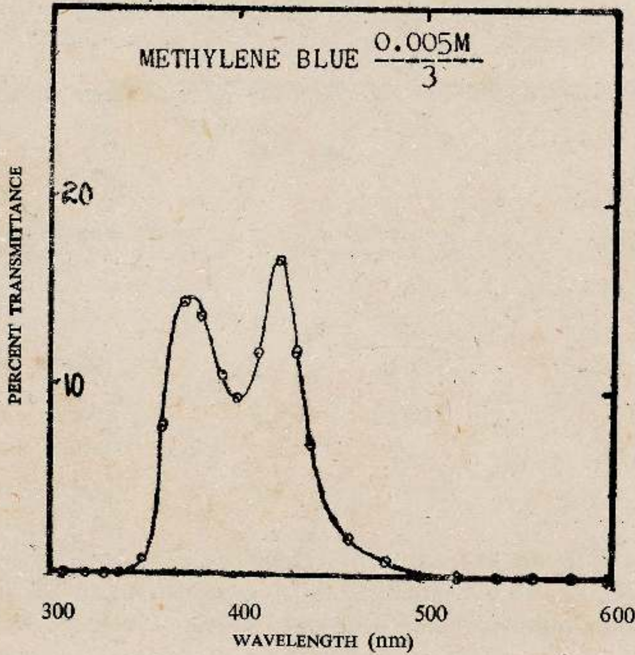

Figure 4

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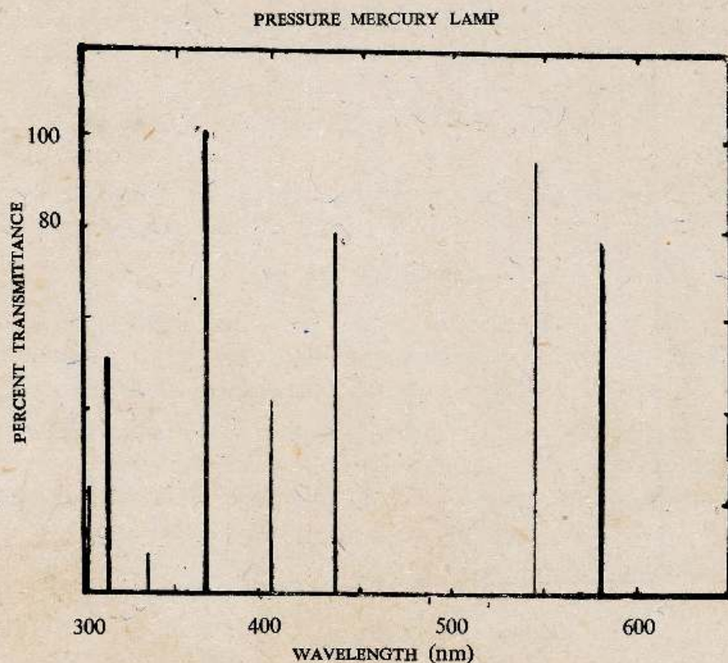

Figure 5. Relative energy distribution in Medium Pressure Mercury Lamp

Transmittance characteristics of the following mixtures were studied (Figure 6).

TABLE 1

Mixture	*Concentration of chrome alum* (*moles* l^{-1})	*Concentration of methylene blue* (*moles* l^{-1})
I	0·28	0·0016
II	0·28	0·0008
III	0·28	0·0005
IV	0·14	0·0006

Transmission spectrum of mixture I (Figure 6) shows 1·5% transmission at $\lambda = 366$ nm. Transmission characteristics of mixtures II and III show that the reduction in concentration of methylene blue causes a hypsochromic shift.

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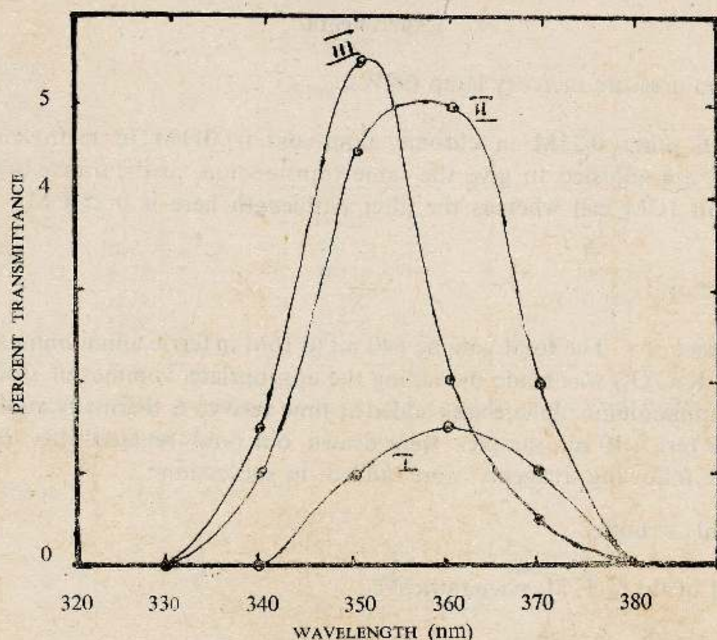

Figure 6

It is easily seen (Figure 7) that mixture IV (0·14M in chrome alum and 0·0006M in methylene blue) constituting a monochromatic filter has a transmittance of 11% at 362 nm. Thus, we have achieved a near perfect monochromatic filter for 366 nm.

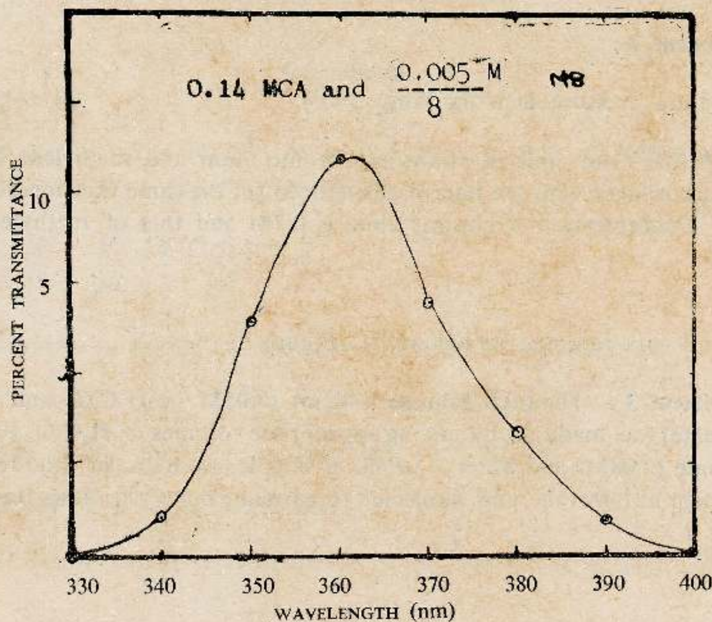

Figure 7

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4. Experimental

Lamp : medium pressure mercury lamp (90W).

Filter : 'CAMB' filter 0·25M in chrome alum and 0·0011M in methylene blue, concentrations are adjusted to give the same transmission, as the transmission was measured in an 1CM cell whereas the filter pathlength here is 0·55 CM.

Reactor : Reactor I

4.1. Experiment 1 : The total volume 640 ml (0·15M in ferric ammonium sulphate and 0·06M in $K_2C_2O_4$) was made by adding the appropriate volumes of stock solutions. Ferric ammonium sulphate was added at time zero to a thermally equilibrated mixture of the rest. 10 ml samples were drawn out (and replaced by 10 ml of water) and the following reagents were added in succession ;

(i) 10 ml of buffer,

(ii) 2 ml of 0·1% 1, 10 phenanthroline

and the total volume was made up to 50 ml.

The resulting mixture was subjected to the analysis as described by Calvert and Pitts.[2] Photosensitive solutions were handled in the dark. (Temperature at which the experiment was done = 29°C ± 1°C).

4.2. Experiment 2 :

Lamp : Medium pressure mercury lamp. (90W)

Filter : "CA/MB" but concentrations of chrome alum and methylene blue were adjusted in accordance with the filter pathlength to get the same transmission as with 1 CM cell. Concentration of chrome alum is 0·7M and that of methylene blue is 0·006M.

Experiment 1 was repeated in reactor II (Figure 2).

4.3. Experiment 3 : The total volume 640 ml (0·05M in $H_2C_2O_4$ and 0·01M in uranyl sulphate) was made up by mixing appropriate volumes of $H_2C_2O_4$ (0·8M) and uranyl sulphate (0·08M) and water. 10 ml. of samples were drawn (and replaced by 10 ml of water) at intervals and subjected to permanganate titrations (temperature 30°C ± 1°C).

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5. Results

5.1. Experiment 1 :

TABLE 2

No.	*Time (min)*	*O.D. (optical density)*	*corrected optical density*
1	3.17	0·055	0·055
2	10.50	0·175	0·178
3	14.00	0·245	0·253
4	18.00	0·265	0·278
5	23.00	0·295	0·315

5.2. Experiment 2: The reaction was immeasurably fast and optical density measurements were impossible.

5.3. Experiment 3 :

TABLE 3

No.	*Time (min)*	*Vi (ml)*	V_i^{corr} *(ml)*
1	0	103·70	103·70
2	20	102·10	102·10
3	40	99·00	100·58
4	60	96·00	99·07
5	80	92·80	97·35
6	100	89·30	95·28

6. Calculation

6.1. Experiment 1 :

The appropriate equation for the evaluation of light flux or intensity output emitted by the lamp filter system is given by

$$I = \frac{50}{10} \cdot \frac{V}{\phi \varepsilon l} \left\{ \frac{d\,(O.D.)^{corr}}{dt} \right\} \tag{1}$$

where I is the intensity output by the lamp filter system,

ϕ : is the quantum yield of the actinometer reaction,

ε : is the extinction coefficient of $Fe(phen)_3{}^{2+}$ at 510 nm,

l : pathlength of absorption cell, and

V : total volume in litres, when the results are subjected to least square treatment.

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$$\frac{d\,(O.D^{corr})}{dt} = 1.68 \times 10^{-2}\ \text{min}^{-1}$$

and substitution of appropriate values into the equation (1) yields

$$I = \frac{50 \times 0.640 \times 1.68 \times 10^{-2} \times 6.023 \times 10^{23}}{10 \times 1.20 \times 1.1 \times 10^{4}}$$

$$= 6.25 \times 10^{18}\ \text{quanta min}^{-1}$$

6.2. Experiment 3 : When the results of Experiment 3 are subjected to least square analysis, we get

$$\frac{dV^{corr}_{KMnO_4}}{dt} = -\,0.068\ \text{ml min}^{-1}.$$

where $V^{corr}_{KMnO_4}$ is the corrected volume of $KMnO_4$ required at time t, with co-efficient of correlation (r^2) = 0.9964 which shows how suitable the results are for linear regression.

The zero time datum gives

$$N^{KMnO_4} = 0.0096\ \text{eq l}^{-1}.$$

$$-\frac{d[OX]}{dt} = \frac{-d\left[V^{corr}_{KMnO_4}\right]}{dt} \cdot \frac{N_{KMnO_4}}{10\ \text{ml} \times 2\ \text{eq mole}^{-1}}$$

The equation for the determination of absorbed intensity (I) is given by the following equation :

$$I = \frac{V}{\phi}\left[\frac{-d[OX]}{dt}\right]$$

$$I = \frac{0.640 \times 0.068 \times 0.0096 \times 6.023 \times 10^{23}}{0.49 \times 10 \times 2}$$

$$= 2.57 \times 10^{19}\ \text{quanta min}^{-1}.$$

7. Discussion

Consider the lamp-filter system of reactor I. Measurement shows that the total thickness of glass through which the light travels before coming into contact with the reaction mixture is 0.62 cm and the filter pathlength is 0.55 cm. Figure 8 shows the transmission characteristics of 0.62 cm thick pyrex glass from which the transmission at 365 nm is 64.8%. Effectively, the relative intensity felt by the reaction mixture is $\frac{64.8}{100} \times 10.25 = 6.6\%$ at 365 nm.

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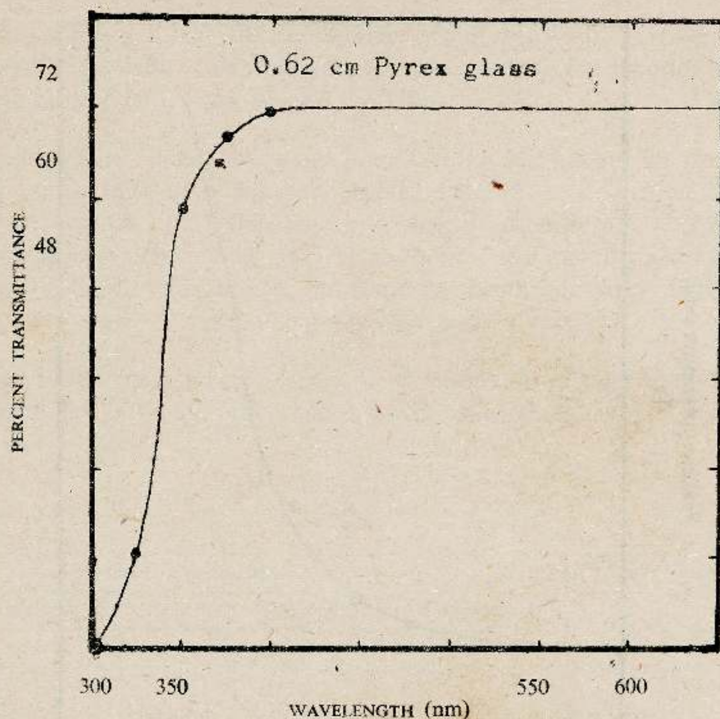

Figure 8

If the percent transmittance of the lamp-filter system was 6.6%, an intensity of 10^{19} quanta min^{-1} order(deduced on the basis of experimental results[4] obtained in previous work at the laboratory) should have been observed. The reduction in intensity was suspected to be due to the interaction of chrome alum and methylene blue at higher intensities as interaction has been shown to be absent[5] at low intensities.

Therefore chrome alum and methylene blue were kept in two separate concentric cylindrical compartments and Experiment 2 was carried out in reactor II. Measurements show that the total thickness of glass through which the light passes before coming into contact with the reaction mixture is 0.78 cm. The transmission characteristics of pyrex glass of 0.78 cm thickness is shown in Figure 9, from which the relative intensity output by the lamp-filter system can be shown to be $\frac{10.25 \times 62}{1000} = 6.35\%$ which is comparable to the relative intensity output (6.60%) of the former filter system (CAMB filter).

In Experiment 2, observation of ferrous oxalate precipitation before any measurements could be made indicates that the reaction was complete in a short period of time. It obviously implies that the intensity has been increased greatly.

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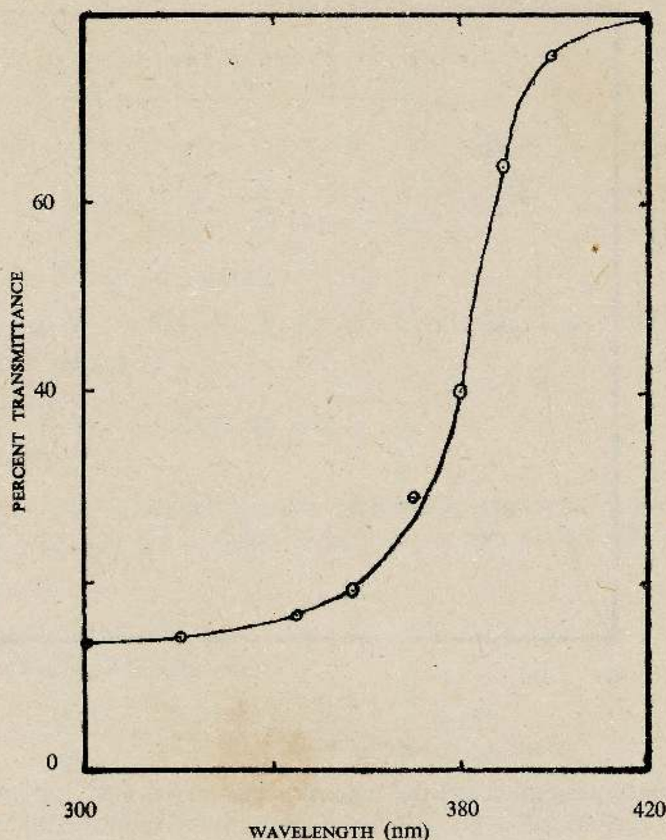

Figure 9

As uranyl oxalate actinometry is most suitable for high flux of light, Experiment 3 was performed, (the results of which are shown in Table 3,) from which the intensity turns out to be 2.57×10^{19} quanta min^{-1}.

The use of two filter compartments results in a four-fold increase in intensity. From this increase in percentage transmission with high analyzing light intensity when chrome alum and methylene blue are isolated from each other, it may be concluded that an association complex is formed between either one or more excited species and ground state species of chrome alum and methylene blue.

Since methylene blue is well known as a photosensitizer,[6] we can cautiously suggest that the first excited triplet of methylene blue is interacting with the Cr^{3+} or in other words, it may be a case of exciplex formation. But what is significant about this is the fact that one of the components is an inorganic species.

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Thus a highly monochromatic lamp-filter system has been achieved emitting a reasonably high intensity. We shall now compare some of the reported values with the intensity output and properties of the filter.

Hammond obtained 3×10^{19} quanta min^{-1} in the 365 nm region from a 800W showing a power utilization efficiency (PUE) of 3.75×10^{16} quanta min^{-1} W^{-1}. 2.57×10^{19} quanta min^{-1} from 90W lamp was obtained showing a PUE of 2.85×10^{17} quanta min^{-1} which is eight times as large as Hammond's and also the light is more monochromatic than in the case of Hammond's experiment, since the half band width is 25 nm, whereas it was 50 nm in Hammond's case.

Calvert and Pitts[1] recommend a filter for the isolation of light at 366 nm. The comparison of this with the filter used is given below (Table 4).

TABLE 4

Calvert-Pitts (Figure 10)		*"CA/MB" filter (Figure 11)*	
Components	*Pathlength*	*Components*	*Pathlength*
1. Components			
(i) aqueous $CuSO_4$ solution 50 gl^{-1}	10 cm	(i) aqueous solution of chrome allum (0.7 mole l^{-1})	0·2 cm
(ii) Corning glass 7-37 (5800)	0·5 cm	(ii) aqueous solution of methylene blue (0·006 mole l^{-1})	0·1 cm
(iii) 2, 7 dimethyl 3, 6 diazo cyclopepta 1,6 diene perchlorate (0·1 gl^{-1})	1 cm		
2. Testing time : 113 h		Length of time is immaterial	
3. Light stability : Increase in transparency observed in the first four hours		No change in transparency	
4. Half band width : 30 nm		Half band width : 25 nm	
5. Maximum transmittance : 25%		6·5%	

The above table shows the relative advantages of the filter over the other except the fact that the maximum transmission in this case is nearly four times less than Calvert's and Pitt's.

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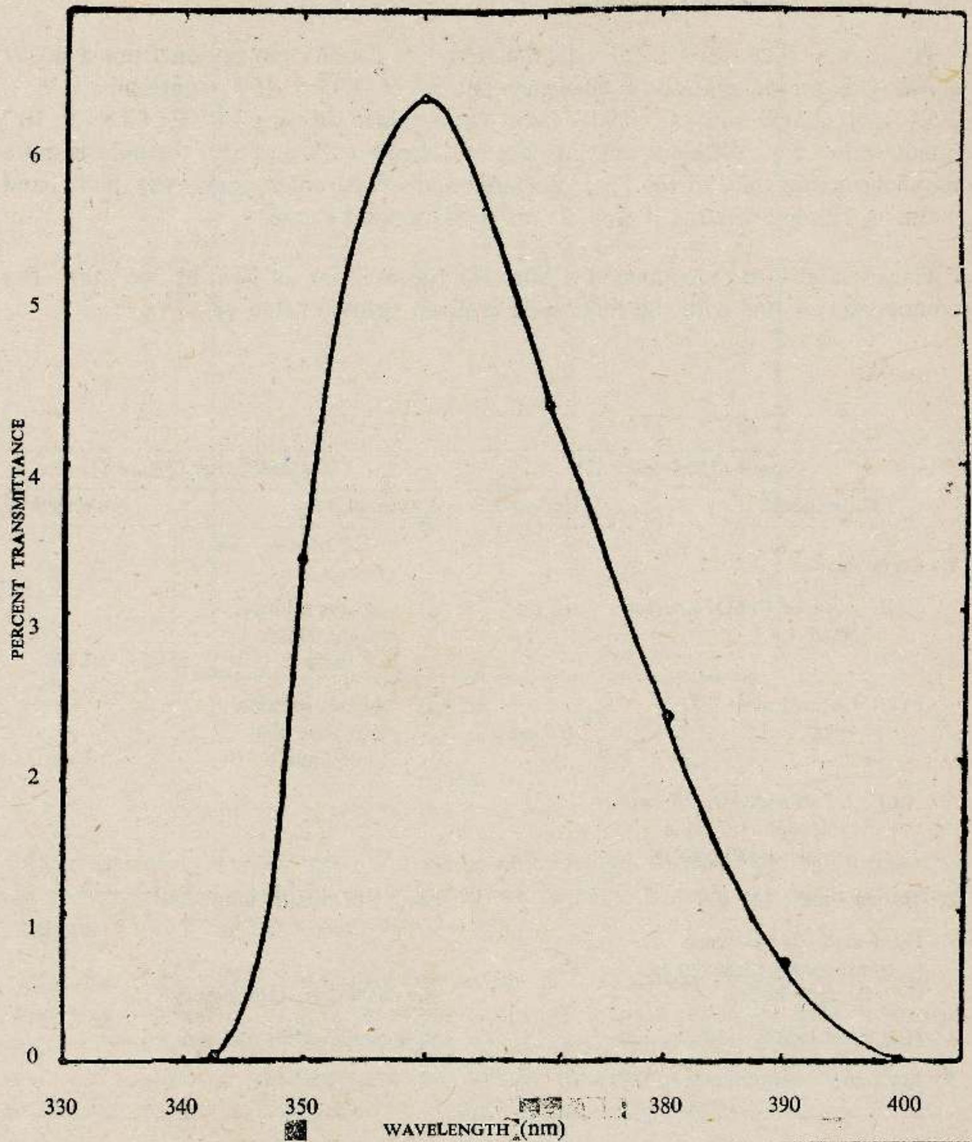

Figure 10. Percent light transmission after passing through the lamp filter system.

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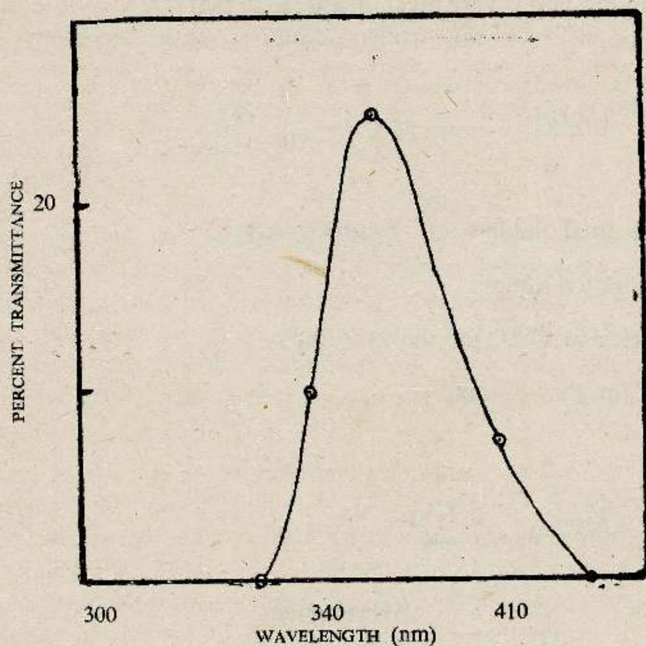

Figure 11. Transmission of filter combination for isolation of 365–366 nm region

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It is expected that further experimentation with relative concentrations of two components will no doubt enable an attainment of a higher percentage of transmittance. Further experimentation in improving the filter and detection and elucidation of the structure of highly absorbing intermediates in the irradiated benzophenone-iso-propanol system are the current main interests.

Addendum

In Experiments 1, 2 and 3, each sample of reaction mixture was replaced by an equal volume of water in order to prevent the error due to the decrease in volume. The error due to dilution is very much smaller than that due to a drop in volume. A correction can be made for this artificial dilution. It can be shown that

$$(O.D_i^{corr}) = \frac{V}{V - \sum\limits_{i=l}(i-1)\nu} O.D_i$$

where V is the total volume of reaction mixture,

ν is the volume of sample,

$O.D_i^{corr}$: corrected $O.D.$ for the ith sample,

$O.D_i$: $O.D.$ for the ith sample.

Similarly

$$V_{i\,KMnO_4}^{corr} = \frac{V}{V - \sum\limits_{i=l}(i-1)\nu} V_{i\,KMnO_4}$$

Abbreviations

1. "CAMB" filter : an aqueous solution containing chrome alum and methylene blue.
2. "CA/MB" filter : aqueous solutions of chrome alum and methylene blue kept in separate compartments.

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Cultivation, Isolation, Purification and some Properties of the Enzyme Glucoamylase from *Aspergillus Niger*

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Abstract: Glucoamylase from *Aspergillus niger* was purified eleven-fold and its properties studied. The preparation was apparently free of α amylase and transglucosidase. Studies on the effect of temperature revealed that although the enzyme begins to be deactivated at 60°C, this temperature would be most effective in the commercial conversion of starch to glucose. An easy method of assay of α amylase levels in culture filtrates is also described. Studies with DEAE-cellulose also revealed the presence of minor glucohydrolases.

1. Introduction

Glucoamylase also known as amyloglucosidase but more correctly named α 1-4 glucanglucohydrolase (3.2.1.3) is a common starch hydrolysing enzyme present in many species of fungi (notably in *Aspergillus*[3,15] and *Rhizopus*[2,4,8] species) and also in certain yeast and bacteria.[5] The enzyme (an exoenzyme) hydrolyses starch in a stepwise manner from the non-reducing termini to produce glucose.[5] In addition to hydrolysing the α 1-4 bond, the enzyme can also hydrolyse the α 1-6 bond[5] (present in amylopectin).

The enzymic reaction is of considerable importance as the conversion is used in the commercial production of glucose.[10] Considerable work has been done on the purification of glucoamylase from many species of *Aspergillii*;[5,9,13] the enzyme has also been crystallised.[16] Studies on the enzyme have shown that its pH and temperature optima vary from strain to strain.[9,10] The enzyme is also reported to exist in the form of isoenzymes which differ in their pH optimum.[13]

Our studies in this paper have been undertaken with a strain (selected as previously described[6]) which produces larger quantities of the enzyme than three type strains of *Aspergillus* (which are noted for producing high yields of glucoamylase) when the strains are compared by growing them on locally available media.[7]

Our studies have been directed towards :

(1) The preparation of a reasonably pure preparation of glucoamylase (from this strain) which is free of transglucosidase (amylo 1-4, 1-6 transglucosidase) and α amylase.

(2) The study of its properties mainly with respect to pH and temperature in order to evaluate the best conditions of hydrolysis of starch to glucose using glucoamylase from this strain of *A. niger*.

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2. Experimental

2.1 Production of enzyme

The medium[1] consisted of manioc starch, 20g ; soya bean meal, 5g ; soya bean meal digest, 5g ; $(NH_4)_2HPO_4$, 1g ; NH_4Cl, 2.5g ; $MgSO_4.7H_2O$, 0.5g ; $FeSO_4$, 10mg ; and KCl, 0.5g ; per litre. The soya meal digest was prepared as follows :—

Fresh soya beans were dried to approximately 5% moisture and ground in an edgerunner mill. The flour thus produced was extracted with petroleum ether (60 to 80) in a soxlet for 24 h, dried and reground into a fine powder. The powder was sieved (100 mesh) and suspended in water (1g for 20ml water). To this suspension was added standard papain (0.2g) and the mixture shaken (on a reciprocal shaker) for 4 h at room temperature (28°C to 30°C). The fungus was grown by innoculating approximately 10^7 spores (of strain CISIR—N_4) suspended in 'Tween 80' (0.1ml 'Tween 80' per 1) into 250 ml Erlenmeyer flasks containing 50 ml of medium. The flasks were then swirled on an eccentric shaker for 5 days at room temperature (28°C to 30°C), after which the fungal pellets were filtered to give a clear filtrate containing the exo-enzyme.

2.2 Purification of enzyme

Glucoamylase was purified using the standard methods of protein purification, namely, solvent precipitation, salt precipitation, Sephadex gel chromatography and DEAE-cellulose chromatography. Preliminary studies showed that the bulk of the activity was precipitated at concentrations between 70% and 80% isopropyl alcohol. Further, most of the glucoamylase was precipitated at concentrations of $(NH_4)_2SO_4$ between 70% and 90% saturation. This data was used in the purification of glucoamylase. The procedure adopted was as follows: to the crude filtrate was added isopropyl alcohol to 80% by volume and the mixture left to stand at 0°C for 4 h to precipitate the enzyme. The protein was separated by centrifugation and dissolved in a few ml 0.04M acetate buffer pH 5.5 and introduced into a column of Sephadex G-200 (bed volume 92 ml). The mixture of proteins was eluted at 20 ml/h with 0.04M acetate buffer pH 5.5 collecting 2 ml fractions. The fractions containing saccharogenic activity was pooled and concentrated by $(NH_4)_2SO_4$ precipitation (overnight at 0°C) retaining the protein precipitated between 70% and 90% saturation. The precipitate was dissolved in 0.02M acetate buffer, pH 5.5 and was dialysed against the same buffer (three times with 800 ml buffer). The dialysate was introduced into a column of DEAE-cellulose (40 ml bed volume equilibrated in 0.02M acetate buffer pH 5.4) and eluted using the same buffer containing the following concentration of NaCl, (*a*) No NaCl (50 ml) ; (*b*) 0.1M NaCl (15 ml) ; 0.15M NaCl (15 ml) ; 0.20M NaCl (15 ml) ; 0.25M NaCl (15 ml) ; 0.3M NaCl (30 ml) ; 0.4M NaCl (50 ml) and 0.5M NaCl (80 ml) at an elution rate of 20 ml/h while collecting fractions of 3 ml and 2 ml.

2.3 Measurement of activity

Total saccharogenic activity was measured by quantitating the glucose released by the method of Nelson.[12] The reaction mixture contained 7.5 ml starch (2%), 1 ml of 0.2M acetate buffer (pH 4.3) and sufficient quantity of enzyme (in 0.1 ml). The mixture was incubated at the specified temperature (generally 55°) for 1h, aliquots (0.1 ml to 0.3 ml) being removed at zero time and at 15 min intervals. The aliquots were introduced into the Nelson reaction mixture which was immediately placed in a

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boiling water-bath. The colour developed was measured using a Klett Summerson Colorimeter (green filter). The amount of glucose liberated at various times was calculated using a standard curve. Enzyme activity, expressed in μ moles/ml/min, was calculated using the progress curve of the reaction (which was generally linear). Corrections were made for protein by using blank and zero time readings which are subtracted from reducing sugar volumes. It was found that these values are very low ($<$ 5 Klett) due to the small amount of protein present in comparison to the experimental readings which were in the order 50 to 225 Klett.

2.4 Properties of the enzyme

The effect of pH and temperature on enzyme activity were studied using the above conditions using a thermostated water bath and a buffer (acetate) of the appropriate pH (0.4M). Reaction time was varied according to the purpose of the experiment (30 min or 20 h).

2.5 Starch-iodine reaction

The reaction was performed by the standard method[14] using 1 ml of 1/10 diluted reaction mixture and reading the absorption at 600 nm on a UNICAM SP3 spectrophotometer.

2.6 Estimation of protein

This was done by the method of Lowry.[11]

2.7 Chromatography

Paper chromatography of reaction products was performed using Whatman No. 1 chromatography paper with butanol : pyridene : water (4 : 6 : 3) as solvent.[16]

3. Results and Discussion

3.1 Some properties of the crude extract

The supernatant resulting from 5 days fungal growth was a dark pink easily filterable liquid of pH 6 to 7. It generally contains a total saccharifying activity equivalent to about 40 μ moles glucose/ml/min which is mainly the result of glucoamylase activity. However, varying amounts of α amylase were also present. This was especially evident about a day before the optimum period of growth for glucoamylase production.

Evidence for α amylase activity was the rapid clearing of starch without liberation of sufficient quantities of reducing sugar. The variation of α amylase levels of cultures is shown in Figure 1. The decrease in absorption (at 600 nm) of the starch-iodine reaction is proportional to α amylase content. The percentage decrease in absorption when 20% of the starch is converted to glucose gives a good estimate of the α amylase activity of the extract.[6] This may be given a numerical value by plotting a standard curve with known ratios of α amylase and glucoamylase (Figure 2). The α amylase used in this experiment was BDH α amylase and the glucoamylase used was a crude extract incubated at pH 2.5 for 4 days at 0°C to destroy α amylase. Although this gives a good estimate of α amylase levels it is not strictly valid because: (1) BDH α amylase (bacterial) and not the α amylase from this *A.niger* strain was used and (2) experiments carried out later showed that the glucoamylase used probably had low levels of α amylase (compare with Figure 5).

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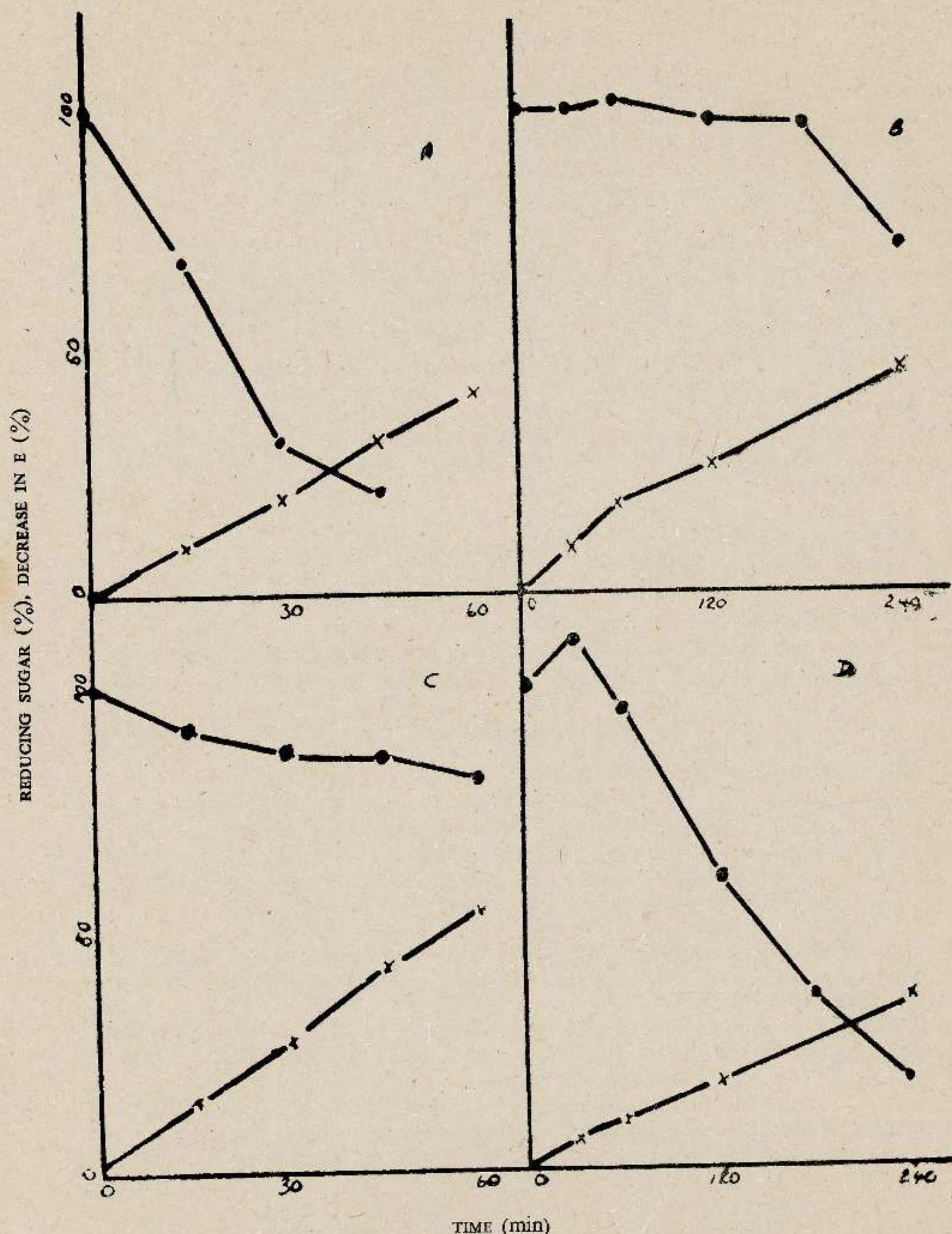

Figure 1. α Amylase levels of culture filtrates

Insets, A, B, C and D show the % release of reducing sugar and % decrease in absorption (E) at 600 nm of the starch iodine colouration by saccharifying enzymes of different culture filtrates. For details see section 3.1.

According to the curve on Figure 2, the α amylase index of A, B, C, and D would be > 30, 0, < 5 and 30 respectively.

X—X, starch iodine colouration.

●—●, glucose released.

Zero time absorption of the starch-iodine reaction was 0.60 (the assay was carried out as in 2.5). This represents 100% absorption. Total reducing sugar releasable was 400 μmoles.

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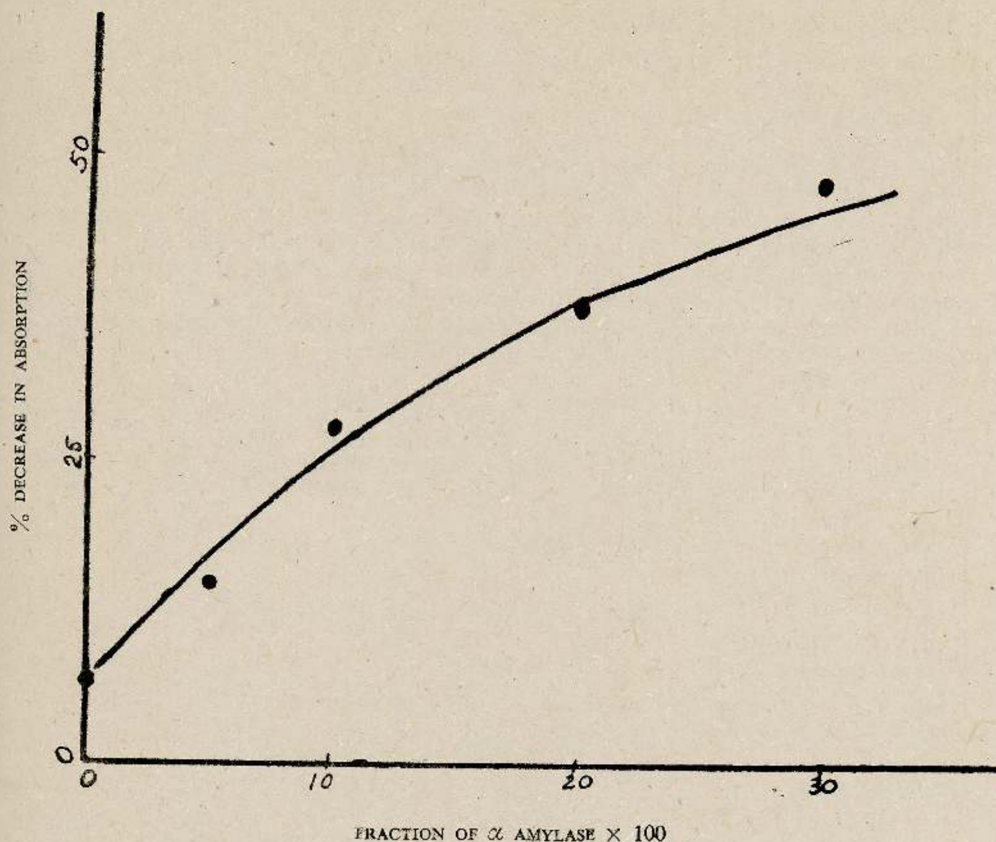

Figure 2. Standard curve for α amylase index.

Percentage decrease of absorption at 600 nm at 20% recovery of reducing sugar (20 D.E.) is plotted vs fraction of α amylase. The activity of α amylase used for the plot being calculated on the release of reducing sugar from starch.

Zero time absorption of the starch-iodine reaction was 0.60 (the assay was carried out as in 2.5). This represents 100% absorption. Total reducing sugar releasable was 400 μmoles.

The saccharogenic activity of crude extracts had a pH optimum that was broad and varied between pH 4.0 and 5.0. The temperature optimum lay between 52°C and 56°C. The extract converted starch to reducing sugar at an efficiency of 85% to 90%. This reflected some transglucosidase activity, which was confirmed by observing the products of hydrolysis by paper chromatography where, in addition to glucose (95% of reducing sugar as determined by the Nelson method after elution of spot) small quantities of other sugars were also present, their R_f values corresponding to that of isomaltose, isomaltotriose and a pentasaccharide.

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3.2 Purification of Glucoamylase

During the preparation of glucoamylase for commercial use[7] the procedure used was to concentrate the enzyme at temperatures less than 55° C (under vacuum) followed by dialysis to remove fungal by-products. The process (Table 1) resulted in a sizable loss of activity. Results in section 3.3.3 indicate that the loss of activity might be reduced by evaporation in the presence of starch. Results in Table 2 show that $(NH_4)_2SO_4$ precipitation and precipitation with isopropyl alcohol were more efficient methods of concentration. However, the commercial application of these methods will largely depend on recovery and re-use of the precipitants. The methods used resulted in an eleven-fold purification of the enzyme (Table 2). Although this appears rather low in comparison with the degree of purification achieved for other enzymes, it must be remembered that glucoamylase is an exoenzyme and is present in a relatively high specific activity even in the crude filtrate.

TABLE 1. Concentration of saccharifying activity.

Step	Total Activity (μmoles/min)	Volume (ml)	Total protein (mg)	Sp Activity (μmoles/mg protein/min)	Fold purification	Recovery (%)
1. Crude Extract	2520	100	102.5	24.5	1	100
2. Concentrate (Vacuum evaporation at 55°C)	1375	27.5	68.8	19.1	0.8	55
3. Isopropyl Alcohol precipitation (80%)	1306	10.5	46.2	28.0	1.14	51

Steps 1, 2 and 3 were carried out successively on the same sample.

TABLE 2. Purification of glucoamylase

Stage of Purification	Total Activity (μmoles/min)	Total protein (mg)	Volume (ml)	Sp Activity (μmoles/mg protein/min)	Fold purification	Recovery (%)
1. Crude extract	1314	168	71.0	7.7	1	100
2. Isopropyl alcohol precipitation	1095	35.2	8.7	31.4	4.1	87
3. Sephadex G-200 Gel chromatography	500	8.0	20.0	61.1	8.0	39
4. Salt precipitation $(NH_4)_2SO_4$	393	5.1	5.7	78.4	10.2	31
5. DEAE-cellulose chromatography	139	1.64	8.0	85.0	11.0	10

For experimental details see section 2.2.

Another point of note is that DEAE-cellulose chromatography (Figure 4) results in two minor peaks of saccharogenic activity. Paper chromatographic analysis of the products showed that glucose was the only reducing sugar formed when starch was hydrolysed. However the specific activity of the peaks at eluent volumes of 119 ml and 111 ml were only 9 μmoles and 7 μmoles glucose/mg protein/min, respectively, and the significance of this existence is not known, but it could indicate the presence of isoenzymes[13] as postulated previously.

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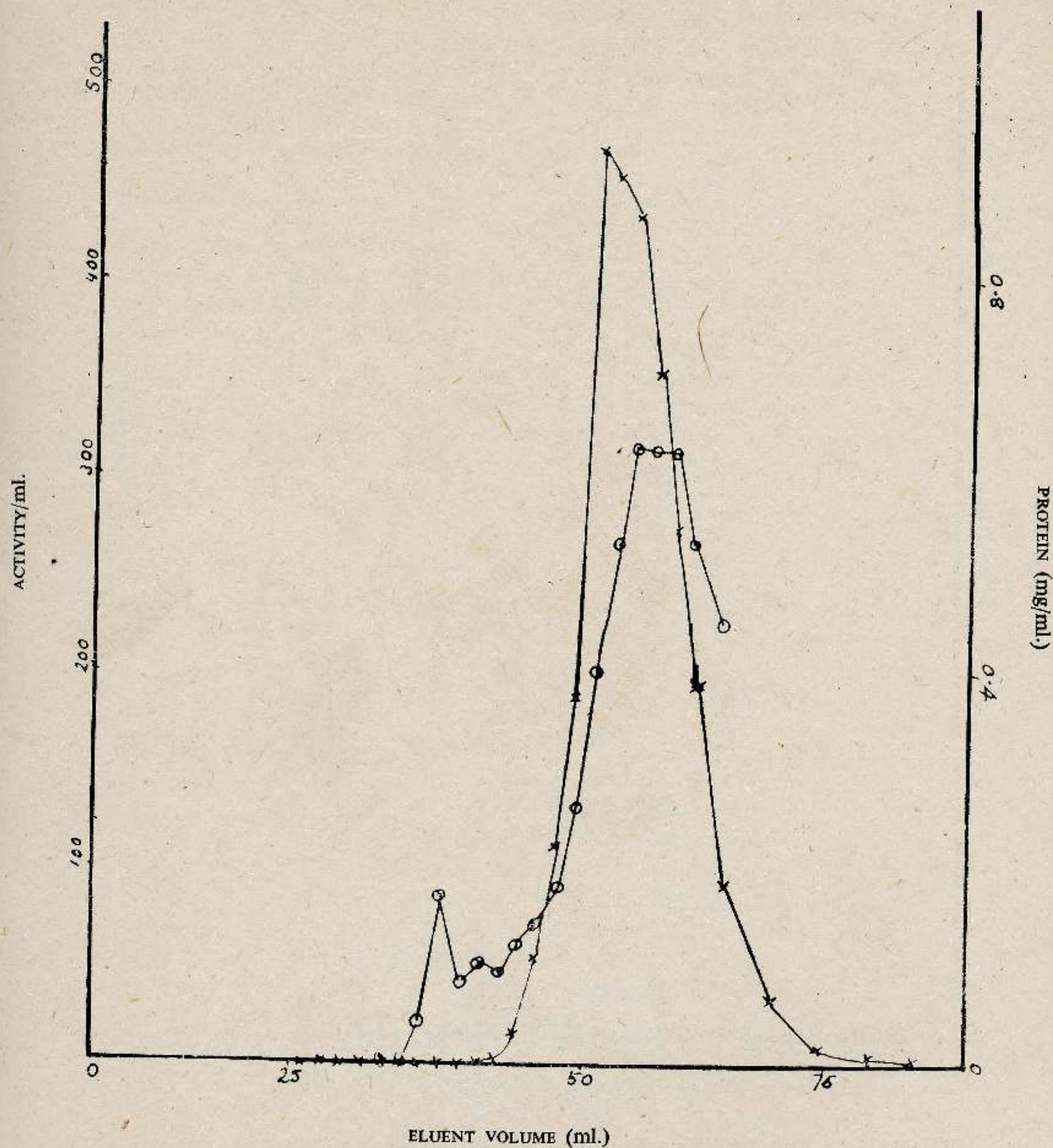

Figure 3. Sephadex-gel chromatography (G-200) of saccharifying activity of *A. niger* filtrates. For details see section 2.2.

O—O, protein
X—X, activity

Activity expressed in mg glucose/ml/h = μmoles/ml/min x 1/10.8.

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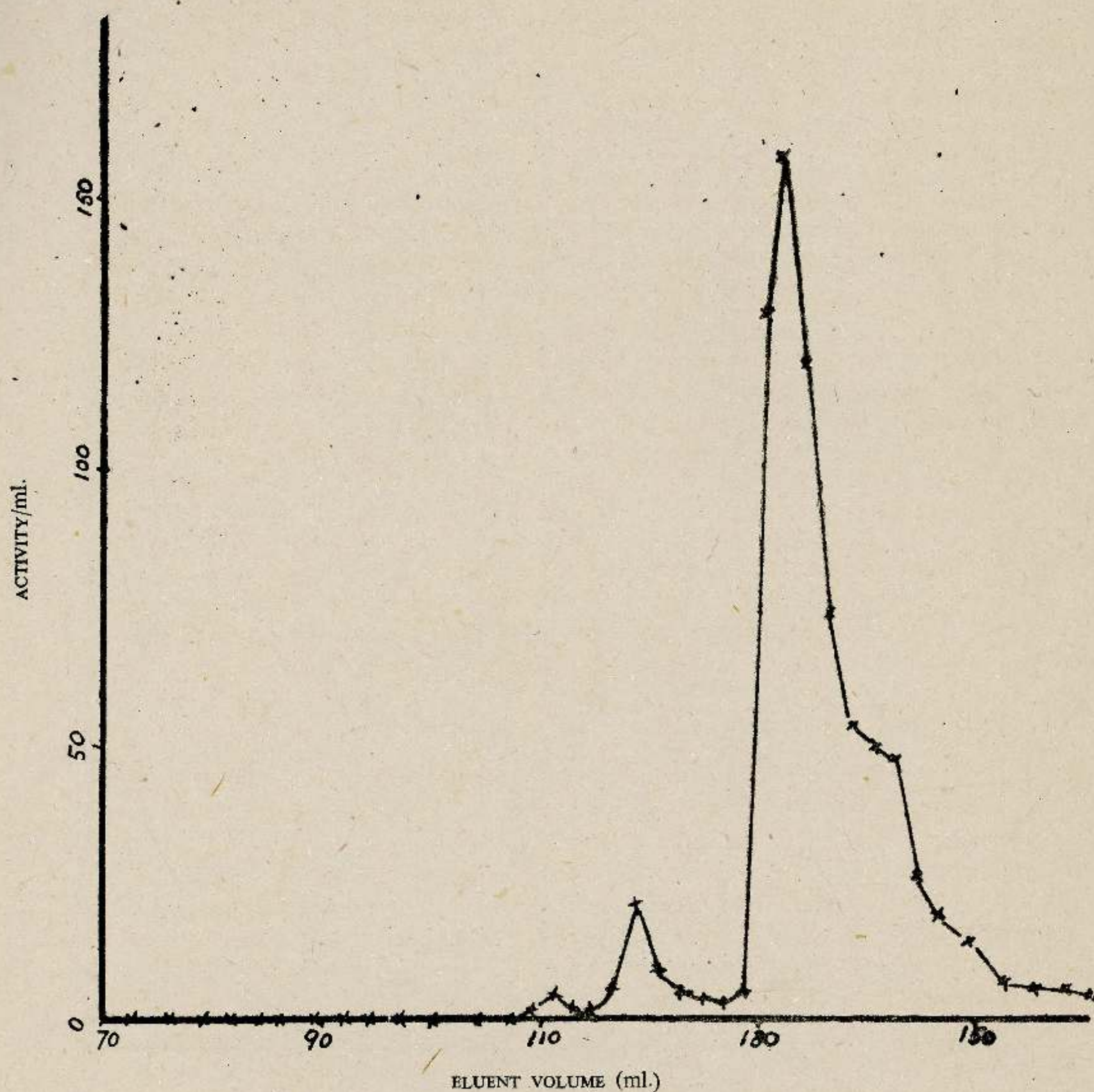

Figure 4. DEAE-cellulose chromatography of glucoamylase containing extracts.

For details see section 2.2.

Activity expressed in mg glucose/ml/h = μmoles/ml/min x 1/10.8.

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Thinning of starch by extracts (reflecting α amylase activity) was observed up to the DEAE – cellulose stage of purification with glucoamylase activity but was lost after this stage probably due to deactivation of α amylase or its irreversible binding on the column.

3.3 Properties of Glucoamylase (purified)

3.3.1 *Purity*

The enzyme preparation did not produce a significant thinning of starch (as evidenced by the starch-iodine reaction) even after more than 40 % of the total theoretical amount of glucose was produced showing that it was free of α amylase (Figure 5). A study of a time course of the reaction (Figure 6) showed that a nearly theoretical amount of glucose (> 97 %) was produced from starch. This indicates the complete (or nearly complete) absence of the transglucosidase in the presence of which yields of this magnitude cannot be achieved.[9] Further evidence for the absence of transglucosidase was provided by paper chromatography experiments which showed that glucose was the only product of starch hydrolysis.

3.3.2 *Basic Kinetics*

The effect of concentration of enzyme (Figure 7) did not have any deviations from linearity showing the absence of complicating factors such as the presence of activators or association-dissociation phenomena. Figure 6 also shows the linear nature of the reaction up to nearly 75 % conversion of starch to glucose.

3.3.3 *Effect of temperature*

An Arrhenius plot of the enzyme activity with temperature is shown in Figure 8 ; the rates being calculated over 30 min reaction time. The plot clearly illustrates the interplay between two opposing factors, (1) the increase in the rate of reaction with temperature and (2) the increase in extent of denaturation with temperature. The figure shows that even over reaction time of 30 min that some deactivation occurred at 60°C (although the absolute reaction rate became progressively faster even up to 70°C). However, a reaction time of the order of 30 min is of little significance for the commercial process which generally needs 20 h to 100 h enzymic conversion. Results on Table 3 give the extent of reaction at varying temperatures over a reaction time of 20 h. Substrate is not limiting and therefore any deviation from the theoretical rate (calculated from extrapolation of linear slope of the Arrhenius plot) is due to deactivation of the enzyme. The results clearly showed that : (1) over this reaction time the optimum temperature is 60°C and (2) at temperature of 55°C and over there is considerable denaturation which progressively increases with temperature. If a calculation of percent denaturation over 20 h is computed, then it is clear that during successive 20 h periods that lower reaction temperatures will

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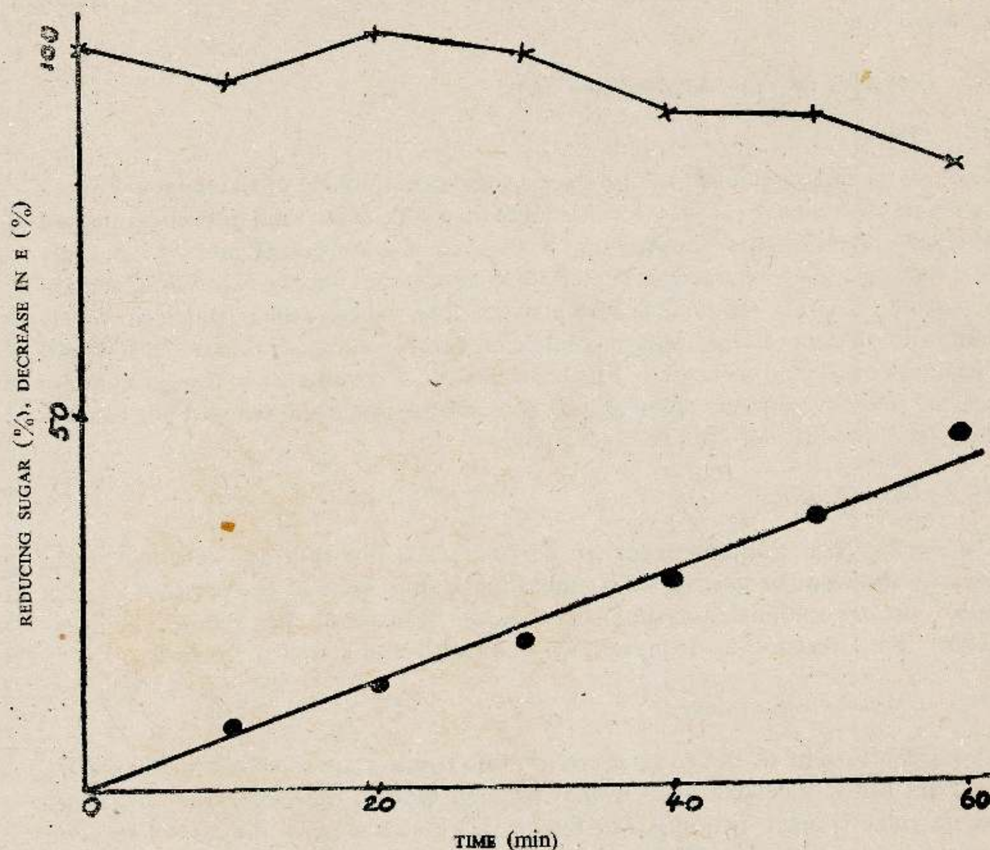

Figure 5. Effect of glucoamylase on the starch-iodine reaction.

Details as in Figure 1 and section 3.1. The reaction mixture was the same as in Figure 6 but the glucoamylase added was ~ 3 μmoles/min contained in a volume of 1.5 ml.

X—X, starch iodine absorption (% of zero time).

●—●, glucose released (% theoretical of starch used).

Zero time absorption of the starch-iodine reaction was 0.60 (the assay was carried out as in 2.5). This represents 100% absorption. Total reducing sugar releasable was 400 μmoles.

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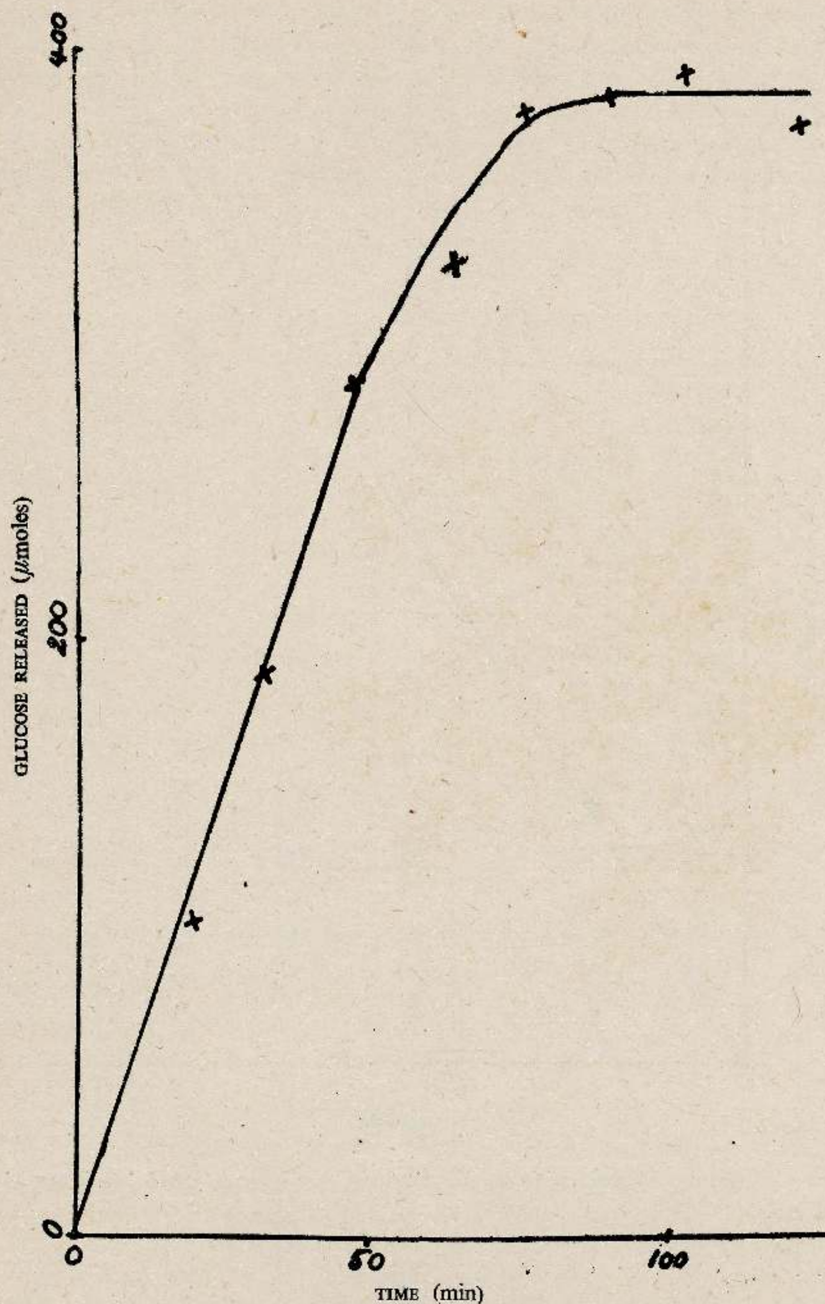

Figure 6. Glucose liberation by glucoamylase.

The reaction mixture contained: 1% starch (7.5 ml) 0.4M acetate buffer, pH 4.3 (1 ml) and glucoamylase (~6 μmoles glucose/min in 0.25 ml). Total theoretical yield of glucose was 407 μmoles

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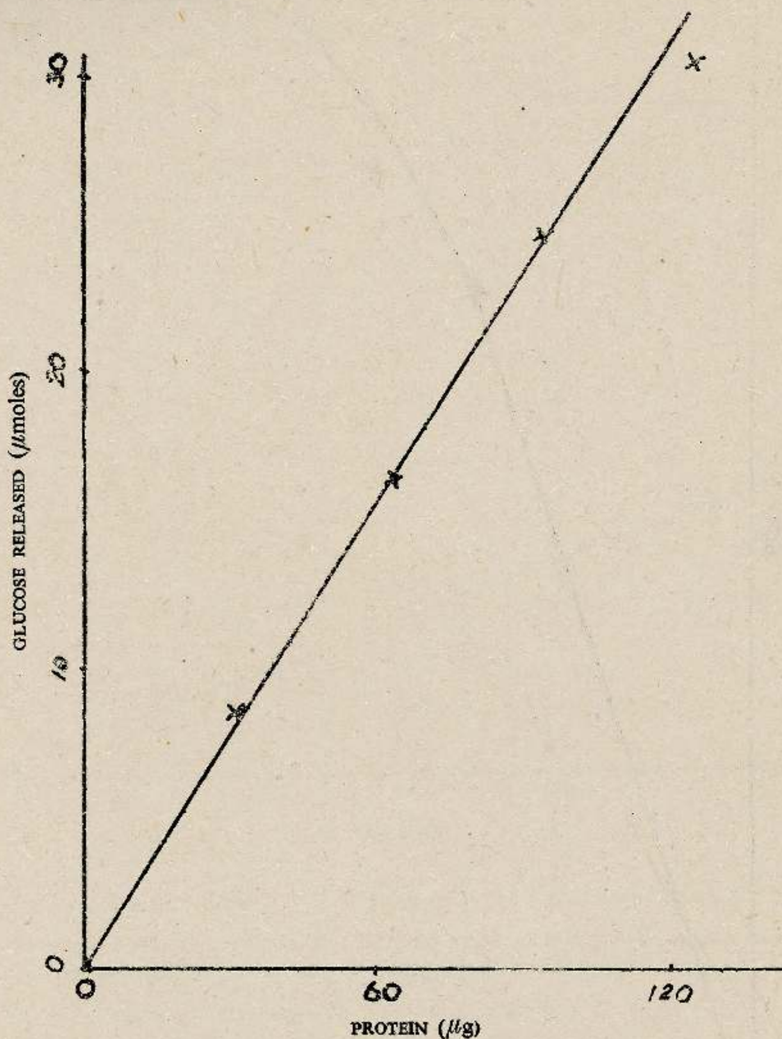

Figure 7. Effect of concentration on the release of glucose from starch by glucoamylase.

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become progressively more favourable. Therefore it would be possible to calculate the most favourable temperature of reaction for different reaction times, keeping in mind that there is a limit to lowering temperature as reduced temperature would favour microbial contamination and deterioration. From these results, it appears that a reaction temperature of 60°C would be most favourable if reaction time is of the order of 20 h to 60 h. However, beyond this reaction time a lower temperature (closer to 50°C) could give a better overall rate of reaction.

TABLE 3. Effect of temperature on hydrolysis of starch

Temperature (°C)	Glucose released (mg)	Theoretical glucose (mg)
28	11	20
38	34	34
45	56	64
50	101	98
55	110	152
60	146	202
65	21	304

Experimental glucose release was estimated by using a reaction mixture containing 2% starch (35ml) 0.4M, pH 4.3 acetate buffer (2 ml) and approximately 0.8 μmoles/min glucoamylase.

Theoretical glucose has been calculated using an extrapolation of the Arrhenius plot of Figure 8.

3.3.4 *Effect of pH*

Unlike the effect of temperature the effect of pH does not appear to be critical. Over both reaction times (30 min and 20 h) the activity is relatively high over a wide range of pH. The optimum pH appears to be 4.0 to 4.3 very little difference occurring between pH 3.6 and 4.6. These studies indicate that the enzyme studied probably corresponds to one of the two isoenzymes isolated by Pazur and Okada[13] (which is reported to have a pH optimum of 4.0 ; the other having an optimum pH of 6.5 according to that study).

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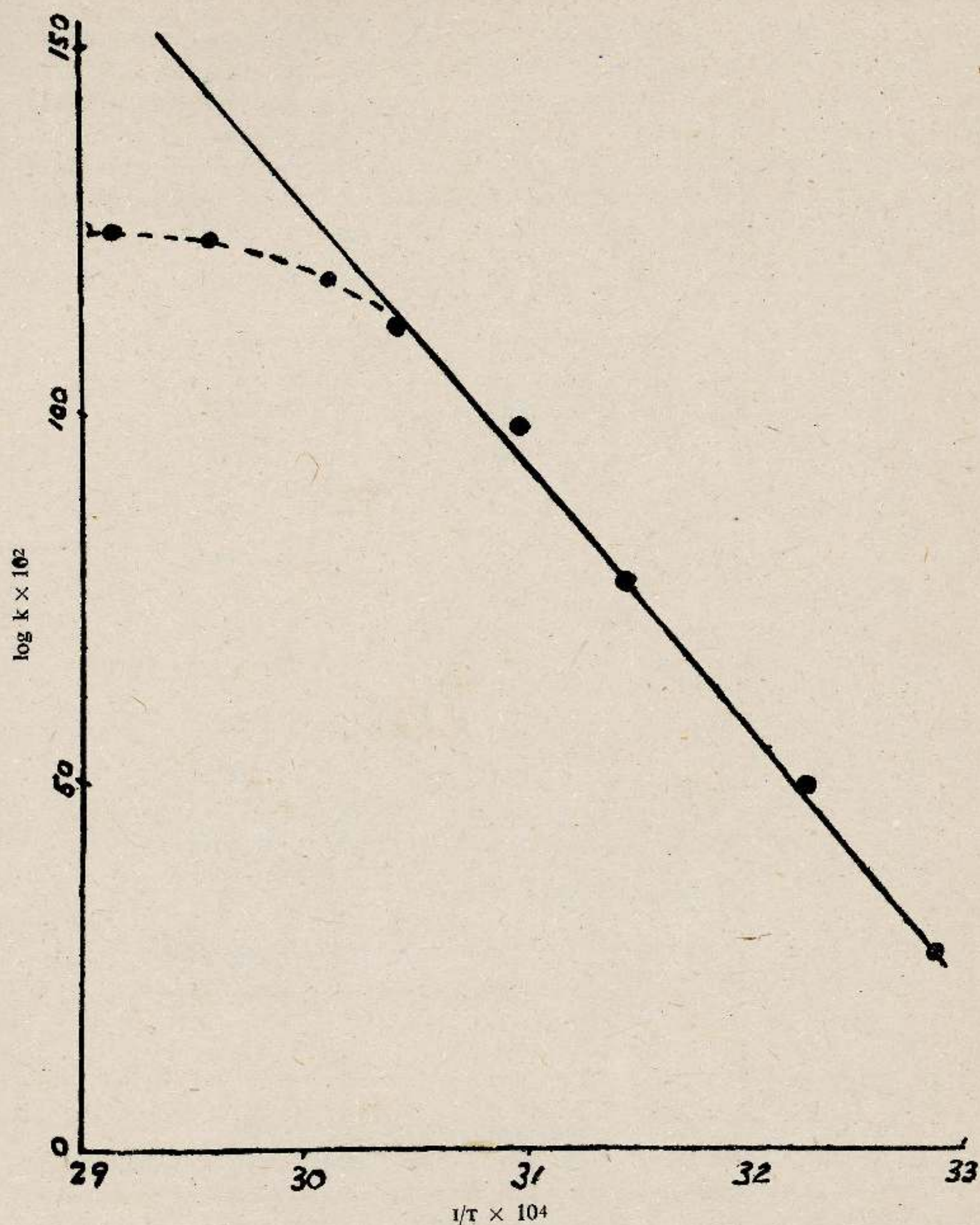

Figure 8. Arrhenius plot for Glucoamylase.

Reaction time was 30 min. K is expressed in terms of μmoles glucose released/ml/min. The extract contains 205 μg protein/ml.

For further experimental details see section 2.3.

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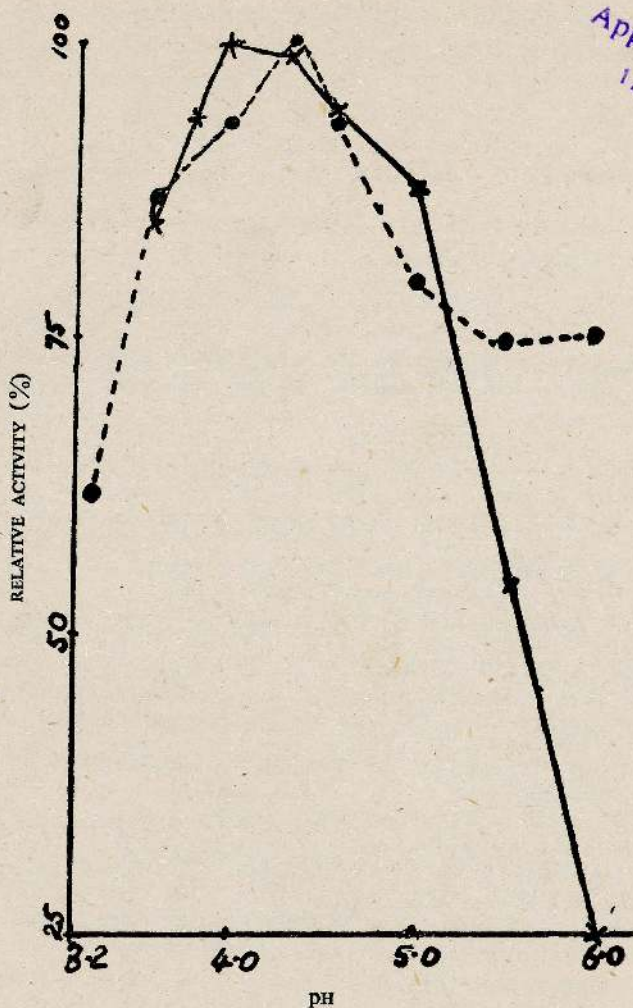

Figure 9. Effect of pH on Glucoamylase activity.

O—O, Reaction time 30 min. Experimental procedure as in section 2.3.

X—X, Reaction time 20h. Experimental procedure as in table 3, but pH varied and temperature constant at 60°C.

100 percent activity represented 17.4 and 18.2 μmoles/mg protein/min. respectively for the experiments.

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4. Conclusions

The glucoamylase produced by a strain of *Aspergillus niger* (producing the enzyme in large quantities on locally available substrate) would be most effective, in the commercial conversion of starch to glucose, at pH 4.3 and 60°C even though there is significant enzyme deactivation at this temperature.

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Effect of Anti-immunoglobulins on Rabbit Peripheral Blood Lymphocytes

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Abstract: The effect of anti-immunoglobulin sera on rabbit peripheral blood lymphocytes was investigated. The lymphocytes with detectable immunoglobulin on the surface showed capping, loss and regeneration of the membrane bound Ig. Following anti-immunoglobulin stimulation, transformation into blast cells was observed.

1. Introduction

Treatment of lymphocytes with "anti-immunoglobulin" sera can cause redistribution of the membrane bound immunoglobulin (Ig)[28] and stimulation of B cells as measured by incorporation of ^{3}H-thymidine.[25] With mouse lymphocytes, the events that result in redistribution of surface immunoglobulin, following the binding of antibodies, which may or may not be sequentially related can be distinguished as "patch formation", "capping" and disappearance of the immunoglobulin from the surface.[5,16,19,28] Following anti-immunoglobulin induced loss of membrane Ig, rapid reappearance occurs. Elson *et al.*[8] have shown resynthesis to the same level, whereas other studies have demonstrated increased concentrations of the resynthesized immunoglobulin.[6,19] Stimulation of rabbit lymphocytes has been observed with anti-allotype sera,[25] anti-class specific sera[23] and antisera to Fab and Fc fragments.[10]

The discovery of easily detectable immunoglobulins on the surface of B lymphocytes,[21,22] implied that the anti-immunoglobulin induced blast transformation occurred in the B cell population. Fanger *et al.*[10] have shown that the response of rabbit peripheral blood lymphocytes to anti-immunoglobulin was not affected by treatment with antisera to rabbit thymus cells, whereas Concanavellin A (Con A) and phytohemagglutinin responses were eliminated, suggesting that B cells, and not T cells, proliferate in response to anti-immunoglobulins. Daguillard and Prochazkova[4] have correlated the degree of response of rabbit spleen cells to goat anti-rabbit immunoglobulin with the percentage of complement rosette forming lymphocytes. By autoradiography and ^{3}H - thymidine uptake, Elfenbein *et al.*[9] have shown that 70% to 80% of rabbit peripheral blood lymphocytes which respond to anti-allotype antisera were complement receptor bearing. These findings suggest that only a subpopulation of B lymphocytes respond to anti-immunoglobulin.

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Most immunoglobulin induced "capping", pinocytosis and regeneration of membrane bound immunoglobulin have been performed with mouse lymphocytes. In this investigation, attempts have been made to link the phenomenon of anti-immunoglobulin induced transformation of rabbit lymphocytes with membrane perturbation process such as capping and to explore the contribution, (if any), by phagocytic/adherent cells to anti-immunoglobulin mediated B cell transformation.

2. Materials and Methods

2.1. Experimental Animals

Outbred rabbits of either sex were obtained from Goodchilds Bros., Sussex, England.

2.2. Medium

In all the experiments "Eagles Minimal Essential Medium" containing 10% Foetal Calf Serum (FCS), penicillin (200 units/ml) and streptomycin (150μg/ml) was used.

2.3. Peripheral Blood Lymphocytes (PBL)

Rabbit blood obtained by ear bleeding (about 25 ml) was defibrinated by gentle shaking with glass beads (BDH, undrilled, about 25) in 20 ml. disposable "universals", immediately after bleeding and mixed with a solution of 3% gelatin in saline (2 parts of blood : 1 part of gelatin). The mixture was allowed to sediment at 37°C for 45 minutes. The supernatant which contained the lymphocytes was removed carefully and washed with warm medium (two washes, centrifugation at 200 g/10 minutes) and finally with cold medium.

2.4. Antisera to rabbit immunoglobulins

Two anti-rabbit immunoglobulin sera raised in goats (G101/68 and G/1286—73) were gifts from Dr. F. C. Hay, Department of Immunology, Middlesex Hospital Medical School, London.

2.5. $F(ab')_2$ fragment of goat anti-rabbit immunoglobulin antisera

The IgG fraction of G/101—68 antiserum prepared by DEAE-cellulose chromatography was digested with pepsin (substrate : enzyme, 20 : 1) in the presence of 0.01 M cysteine, for 12 hours at 37°C. The digest was first fractionated on Sephadex G-200, in acetate buffer, pH 4.8, 0.1 M, and the fractions of approximate molecular weight 100,000 were pooled and recycled on Sephadex G 200. The second gel filtration gave a symmetrical single peak, which on immunoelectrophoresis showed the presence of two components. The major component, $(F(ab')_2$, was of higher mobility, compared to goat IgG and the minor component was a contamination. Absorption on to DEAE-cellulose, at pH 8.1, 0.002 M phosphate buffer removed this contamination.

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2.6. Normal Goat Immunoglobulin (NGIg)

Normal goat immunoglobulin was obtained from normal goat serum by DEAE-cellulose chromatography, at pH 8.1, 0.005 M phosphate in a batch preparation.

2.7. Conjugation of IgG fraction of goat anti-rabbit immunoglobulin to fluorescein

IgG fractions of G/101—68 and G/1286—73 were conjugated with fluorescein iso-thiocyanate (FITC) by the method of Johnson and Holborow.[15] Protein dissolved in bicarbonate buffered saline, pH 9.5, (20 to 25 mg/ml) was added to FITC (dry powder 20 to 40 μg FITC/mg protein) and mixed on a rotor for 1 hour at room temperature. The free fluorescein was removed by gel filtration on a Sephadex G 25 column.

2.8. Anti-immunoglobulin pulsing of lymphocytes and lymphocyte cultures

Lymphocytes were spun down to a pellet and incubated with IgG fraction of goat anti-rabbit IgG (G/101—68) for 1 hour at 4°C. The absolute amount of "IgG fraction of goat anti-rabbit Fc antiserum" used was given under individual experiments. In the negative controls, cells were treated with an equal amount of normal goat Ig (NGIg). After incubation the cells were washed with medium and cultured for varying time intervals at 37°C.

The pulsed lymphocytes were cultured in sterile Eagles Medium containing 10% heat inactivated FCS at 37°C in 5% CO_2 and 95% air in a CO_2 incubator (National Heinicke Company, U.S.A.). All the cultures were performed in sterile "Falcon" tubes at a cell density of 2×10^6/ml/culture.

2.9. Staining of lymphocytes with FITO conjugates

FITC conjugates were diluted (1 : 10 dilution) with medium containing 15mM NaN_3 and incubated with lymphocytes (0.1 ml diluted conjugate 2×10^6 lymphocytes) at 4°C for 1 hour. Before counting, the cells were washed at least five times with medium containing 15mM NaN_3.

3. Results

3.1. Specificity of the anti-immunoglobulin sera

Antiserum G/101—68 was raised in goats against rabbit Fc, but possessed reactivity against both Fab and Fc determinants. Antiserum G/1286—73 was a goat antiserum to rabbit IgG. Both antisera stained abou' 5% of rabbit thymocytes (G 101/68—5.6% (31/541), G 1286/73—4.9% (27/561), which most probably were B cells.

The proportion of rabbit peripheral blood lymphocytes that stained with the fluorescein conjugated G/101—68 and G/1286—73 are shown in Table 1.

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TABLE 1. Staining of Rabbit Peripheral Blood Lymphocytes with Fluorescein Conjugated Antisera (G101/68 and G1286/73) —Effect of Antiserum Dilution

Antiserum dilution	*Percentage of cells stained* G101/68	G1286/73
1 : 4	69	65
1 : 8	60	42
1 : 16	63	58
1 : 32	54	51
1 : 64	50	40

3.2. Capping and regeneration of membrane bound Ig

3.2.1. *Experiment :* Rabbit lymphocytes were pulsed with the IgG fraction of goat anti-rabbit IgG Fc antiserum (200 μg specific antibody/2 $\times$ 10^6 lymphocytes) for 1 hour at 4°C. In the controls, the cells were treated with NGIg. After incubation, the cells were washed to remove excess antibody and cultured at 37°C in aliquots containing 2 $\times$ 10 lymphocytes/ml/tube for various lengths of time. Samples were removed at different time intervals, washed with medium containing 15 mM NaN_3 and stained with FITC—G/1286—73.

3.2.2. *Results :* Incubation of the pulsed cells at 37°C resulted in capping as determined by immunofluorescence. 100% capping of the immunoglobulin positive cells was never achieved. The highest percentage of cells with caps was observed at 45 min to 60 min at 37°C. After about 3 hours at 37°C, the number of cells with caps gradually decreased. At this time, the caps were extremely small and in some cases fluorescent "dots" were seen inside the cytoplasm of some cells. The cells treated with NGIg did not cap, but almost all the cells were patched, presumably due to the conjugated antibody used for fluorescence. The percentage of stained cells dropped to a minimum (about 20%) at 4 hours of incubation at 37°C and this correlated well with the decline of the number of capped cells (Figure 1).

3.3. Stimulation by IgG and F(ab')$_2$ fragment of anti-rabbit IgG antibody

Aliquots of rabbit lymphocyte suspensions in Eagles Medium (2 $\times$ 10^6 lymphocytes/ml) were pulsed with varying quantities of IgG and F(ab')$_2$ fragment of goat anti-rabbit IgG and cultured for 48 hours to 64 hours at 37°C. After the incubation, the cells were washed with medium containing 15 mM NaN_3 and stained with FITC—G/1286—73. The results are expressed as the percentage of immunoglobulin positive blast cells per total immunoglobulin positive lymphocytes.

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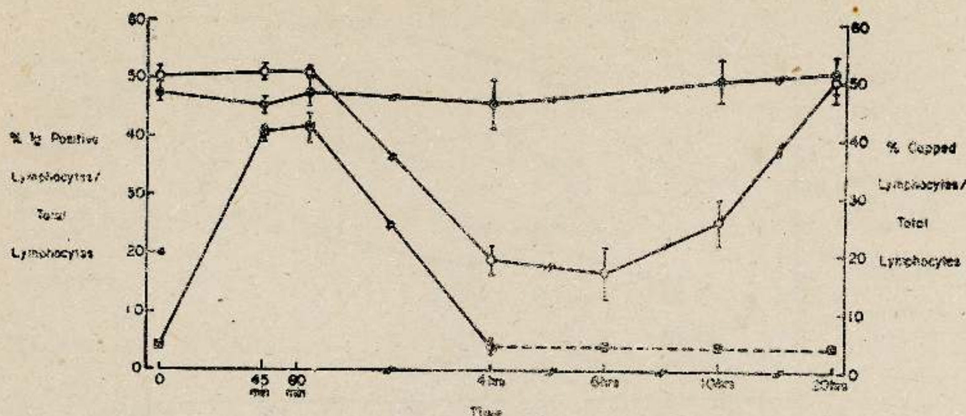

Figure 1. Anti-immunoglobulin induced redistribution of surface immunoglobulin on rabbit peripheral blood lymphocytes.

●—● % $\frac{\text{Ig positive lymphocytes}}{\text{Total lymphocytes}}$, NG Ig treated cells

o—o % $\frac{\text{Ig positive lymphocytes}}{\text{Total lymphocytes}}$, G/R IgG treated cells

■—■ % $\frac{\text{Capped lymphocytes}}{\text{Total lymphocytes}}$, G/R IgG treated cells.

Dose response curves for transformation and capping by the IgG of goat anti-rabbit IgG and for transformation by the $F(ab')_2$ of antibody are shown in Figure 2. The $F(ab')_2$ fragment of the antibody behaved in a similar fashion to IgG of antibody but the blast response was less than that of the IgG antibody (Figure 2).

3.4. Depletion of phagocytic/adherent cells

Rabbit PBL were depleted of phagocytic cells by carbonyl iron treatment and adherent cells by passing through a glass bead column. To lymphocyte suspensions in Eagles medium containing 10% FCS, were added a suspension of carbonyl iron ($Fe_2(CO)_9$), in medium to result a final concentration of 4 mg of carbonyl iron/3×10^6 lymphocytes/ml and incubated at 37°C for 1 hour. These cells which had ingested the iron powder were removed together with the free carbonyl iron by two sedimentations in a strong magnetic field. The supernatants were removed, cells spun down (200 g, 10 min) washed and resuspended in medium containing 50% FCS and filtered through a glass bead column, preheated to 37°C (BDH, Glass Beads for Gas Chromatography, coated with silicone, 10 ml of glass beads/10^8 lymphocytes). The non-adherent cells were collected, washed and resuspended in medium containing 10% FCS. The composition of the phagocytic/adherent cells depleted lymphocytes was determined by neutral red staining.

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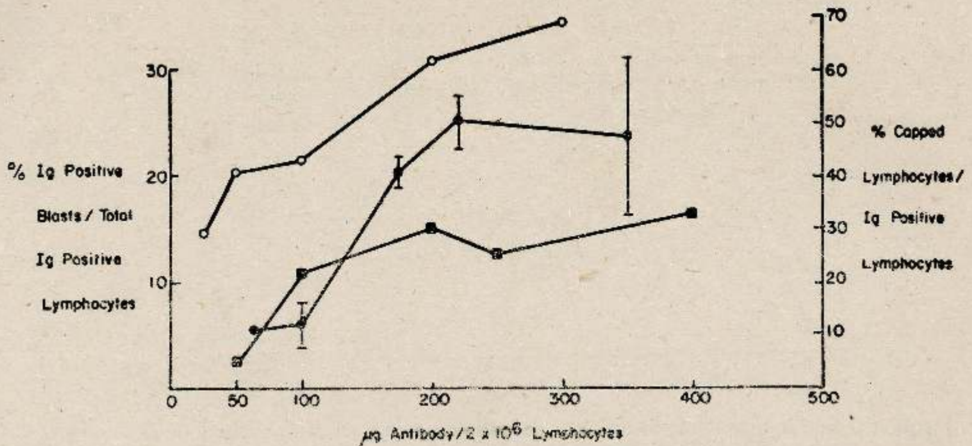

Figure 2. Dose response curves for capping and transformation of rabbit peripheral blood lymphocytes.

o — o Capping by G/R IgG, IgG antibody

● — ● transformation by G/R IgG, IgG antibody.

■—■ transformation by G/R IgG, $F(ab')_2$ of antibody.

TABLE 2. Composition of the Phagocytic/Adherent Depleted Lymphocyte Population

No. lymphocytes, used	3×10^7	3×10^7	7.5×10^7	4×10^7	1.35×10^7	5.6×10^7
No. lymphocytes recovered	1.8×10^7	1.5×10^7	4×10^7	1.9×10^7	7.5×10^7	3.6×10^7
Percentage yield (approximate)	60	50	53	47	55	64
Percentage neutral red positive	1.6	1.1	0.9	0.5	1.2	1.0

(Each column represents results of one experiment)

3.5. Stimulation of rabbit B lymphocytes in the absence of phagocytic/adherent cells

Untreated and adherent cells depleted cell populations were pulsed with goat anti-rabbit IgG (250 to 500 μg antibody/2×10^6 lymphocytes). The negative controls were cells pulsed with NGIg. The pulsed cells were incubated at 37°C for 48 hours to 64 hours, washed with medium containing 15 mM NaN_3 and stained with FITC-G/1286—73.

Depletion of phagocytic/adherent cells reduced the blast responses to a level ntermediate between the response of negative and positive controls. (Table 3).

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TABLE 3. Transformation of Phagocytic/Adherent Depleted Lymphocytes

Lymphocyte population	*Pulsed with*	*Blasts/Total Ig + ve cells*					*Mean ± S.D.*
Normal L	NGIg	12.3 (14/113)	7.7 (6/77)	5.4 (4/73)	4.5 (5/111)	7.4 (10/135)	7.3 ± 3.0
Normal L	G/R IgG	33.7 (28/83)	25 (33/132)	21.6 (22/102)	25.2 (26/103)	19.3 (23/119)	24.9 ± 5.5
Depleted L	NGIg	9.2 (9/97)	3.9 (4/101)	5.7 (6/104)	7.2 (14/193)	5.6 (7/125)	6.3 ± 1.9
Depleted L	G/R IgG	22.3 (21/94)	14.4 (43/297)	7.6 (8/104)	15.6 (16/102)	11.8 (16/133)	14.3 ± 5.4

L = Lymphocytes
Each vertical column represents results of one experiment.
Numbers in parenthesis represent the actual number of cells counted.
(No. immunoglobulin positive blasts/total immunoglobulin positive cells)

3.6. Isolation of phagocytic/adherent cells and composition of isolated cells

Lymphocyte suspensions in Eagle's medium (10^6 cells/ml) were incubated in disposable petri dishes (3ml/petri dish) at 37°C for 30 min. After incubation the non-adherent cells were removed by gentle washing and the adherent cells isolated with the help of the plunger of a disposable injection syringe used as a rubber policeman, the non-adherent cells were further depleted of phagocytic adherent cells by carbonyl iron treatment and glass bead columns. The composition of the isolated adherent cell population was determined by neutral red staining and acridine orange staining.

TABLE 4. Composition of isolated adherent cells

Untreated lymphocytes			
%Lymphocytes	66 (125/189)	73.4 (94/124)	70 (54/77)
%Myeloid cells	33.8 (64/189)	26.5 (34/128)	29.8 (23/77)
Non-adherent lymphocytes			
%Lymphocytes	63.5 (134/211)	83.8 (323/385)	97.1 (137/141)
%Myeloid cells	36.4 (77/211)	16.3 (63/385)	2.9 (4/141)
Isolated adherent cells			
%Lymphocytes	18.4 (26/141)	33.1 (53/154)	20.3 (11/54)
%Myeloid cells	81.5 (115/141)	73.7 (104/154)	79.6 (43/54)

(Each vertical column represents results of one experiment)
(Numbers in parenthesis represent the actual number of cells counted).

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3.7. Stimulation of adherent/phagocytic cells depleted lymphocytes reconstituted with isolated adherent cells

The adherent cell depleted lymphocytes cell population was divided into two parts and one part was reconstituted with the isolated adherent cells. Non-depleted and depleted and reconstituted lymphocytes were pulsed with goat anti-rabbit IgG. In the negative controls, all these cell populations were pulsed with NGIg. The pulsed cells were then washed to remove the free antibody and cultured at 37°C for 48 hours to 64 hours.

Reconstitution of depleted lymphocyte preparations with isolated adherent cells partially restored the stimulation by anti-immunoglobulin. This suggests that either the adherent myeloid cells are involved in anti-immunoglobulin induced B cell stimulation or that adherent lymphocytes which were removed during depletion also contribute to B cell stimulation. The results are summarised in Table 5.

TABLE 5. Transformation of 'Phagocytic/Adherent' Depleted Lymphocytes Reconstituted with Isolated Adherent Cells.

Lymphocytes	*Pulsed with*	*% Blasts/Total immunoglobulin positive cells*				*Mean ±S.D*
Untreated L	NGIg	8.3 (9/108)	1.6 (2/118)	4.6 (6/129)	4.7 (5/106)	4.8 ± 2.7
Untreated L	G/R IgG	27.3 (29/106)	18.5 (21/113)	13.7 (16/116)	27.5 (35/127)	21.7 ± 6.8
Depleted L	NGIg	—	—	3.7 (4/106)	3.4 (4/117)	3.5 ± 0.2
Depleted L**	G/R IgG	6.6 (10/151)	6.8 (8/136)	7.9 (10/126)	7.6 (7/91)	7.2 ± 0.6
Depleted L * Adherent cells	NGIg	—	—	1.7 (2/117)	6.6 (9/135)	4.1 ± 3.5
Depleted L * Adherent cells	G/R IgG	14.2 (16/112)	19.8 (23/117)	9.8 (10/102)	22.3 (27/121)	16.5 ± 5.6

(L = Lymphocytes.
(** cf. Table 3, individual variation, 7.3—22.3)
(Each vertical column represents results of one experiment.)

4. Discussion

Rabbit peripheral blood has been shown to contain a higher proportion of immunoglobulin bearing cells than human or mouse blood. The average distribution is about 20% in man and 27% in mouse.[29] By the mixed antiglobulin reaction, Coombs *et al.*[3] have found wide variation in the number of rabbit lymphocytes bearing IgG, ranging between 24 and 67. Results presented in Table I demonstrate a narrower distribution (40 to 69) in the number of immunoglobulin positive lymphocytes in rabbit peripheral blood, as estimated by immunofluorescence.

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The redistribution of the membrane bound immunoglobulin followed a similar pattern to those described for mouse lymphocytes.[8,28] In contrast to mouse B lymphocytes, 100% capping of the membrane bound immunoglobulin of the immunoglobulin positive cells was not achieved. Greaves and Janossay[12] have quoted a personal communication from Taylor *et al.* that rabbit (B?) lymphocytes, which are very effectively activated by anti-immunoglobulin, cap less readily than mouse immunoglobulin bearing cells.

Both capping and transformation required a minimal concentration of the anti-immunoglobulin antibody, but no stimulation (or relatively little) was observed at the minimal concentration of antibody which resulted in capping (Figure 2). Elson *et al.*[8] observed that antibody concentrations which induced cap formation did not stimulate mouse spleen cells. But an unpublished observation has been quoted that with rabbit lymphocytes, antibody concentrations which capped, also stimulated the lymphocytes.

$F(ab')_2$ fragment of the antibody behaved in a similar fashion to the IgG of the antibody. But the degree of stimulation was less than the IgG antibody (Figure 2). Fanger *et al.*[10] have observed similar behaviour for some $F(ab')_2$ preparations of goat antibody to rabbit IgG Fab and Fc.

In lectin induced transformation of lymphocytes, the removal of the bound lectin at any time upto 18 hours, by adding the appropriate competing sugar was found to diminish the response[18] suggesting that the prolonged or repetitive stimulation or contact of the stimulant is required for activation. The experiments reported here appear to contradict these findings because the excess antibody was washed away after pulsing of the lymphocytes. Figure 1 demonstrates that about 10% to 15% of the immunoglobulin positive cells did not cap, and the surface immunoglobulin (presumably with the bound antibody) persisted up to 20 hours. It would be interesting to know whether these cells that did not cap are the ones that transformed. Furthermore, cytophilic binding of the goat antibody to lymphocytes and myeloid cells could occur, and the elution of these cytophilically bound antibody at 37°C could maintain some free antibody in the culture.

All attempts to differentiate the contribution by phagocytic/adherent cells to transformation from that of cross linkage of the surface immunoglobulin were not entirely successful as all the available techniques for specificially depleting phagocytic cells possess some inadequacies. Methods like carbonyl iron treatment and adherence to glass beads do not result in a specific depletion of a given population and suffer from the disadvantage of poor recovery.

Depletion of phagocytic/adherent cells (Table 2) diminished the blast response by about half (Table 3). If the phagocytic/myeloid cells play a role in anti-immunoglobulin transformation, then one would expect to see a greater reduction in the

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degree of blast transformation. The partial restoration of the response by the addition of isolated adherent cells would imply three possibilities : firstly, that some of the blast forming cells possessing adherent properties were removed during depletion, secondly, that phagocytic/adherent cells were essential for cooperation and thirdly, that both factors were contributory. Some myeloid cell populations are said to possess adherent properties compared to active adherence by macrophages and granulocytes.[26] In general, blast cells and dividing and/or antibody forming cells tend to adhere more than resting small lymphoctes.[13] Absorption of mouse spleen cell suspensions onto nylon wool columns markedly depleted complement receptor-bearing lymphocytes.[2] This taken together with the findings of Daguillard and Prochazkova[4] and Elfenbein *et al.*[9] where complement receptor-bearing cells were said to be responsible for anti-immunoglobulin mediated transformation, provide indirect evidence that the observed reduction in the blast response of the depleted populations was due to preferential removal of the potential blast forming cells. This was supported by the fact that lymphocytes in the isolated adherent cells were transformed with anti-immunoglobulin (Table 6).

TABLE 6. Transformation of Adherent Lymphocytes

Lymphocytes	*Pulsed with*	*% Blasts/Total*	*Ig ± ve cells*
Untreated L	NGIg	3.5 (4/113)	2.1 (2/92)
Untreated L	G/R IgG	21.1 (23/109)	18.3 (18/98)
Non-adherent cells	G/R IgG	5.6 (7/123)	12.8 (13/101)
Non-adherent-carbonyl iron treated	G/R IgG	—	18.0 (17/94)
Adherent cells	G/R IgG	21.0 (20/95)	23.7 (19/80)
Adherent cells-carbonyl iron treated	G/R IgG	21.0 (20/95)	14.1 (15/106)
Adherent cells plus carbonyl iron treated non-adherent cells	G/R IgG	7.3 (7/95)	—

(L = Lymphocytes)
(Each vertical column, results of one experiment)

Are any of the anti-immunoglobulin induced changes observed at cell membrane level directly responsible for stimulation ? In both instances, cross linking of the surface immunoglobulin seems to be a common feature. Fanger *et al.*[10] have shown that bivalent antibody fragments, $(F(ab')_2)$, of goat anti-rabbit IgG Fab/Fc stimulated ^{3}H-thymidine uptake, whereas Fab′ fragments were ineffective in triggering the

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lymphocytes, but inhibited the effect of intact goat anti-rabbit Fab. But when a rabbit antibody to the Fab of goat anti-rabbit Fab antibody was applied, incorporation of ^{3}H-thymidine was induced, suggesting that cross linkage plays an important role in anti-immunoglobulin transformation of lymphocytes. Similarly, in the case of anti-allotype transformation, a second antibody to the allotypic determinants of the first anti-allotype antibody has been said to augment the response.[24]

The phenomenon of capping, *per se*, was not thought to be directly responsible for cell stimulation.[12] A linear co-polymer of glutamine, alanine and tyrosine (GAT) was bound to and induced cap formation from both GAT responder and non-responder mice.[7] Moreover, neither anti-HL-A nor anti-H-2 antisera, which induce cap formation of the corresponding receptors, stimulated lymphocyte proliferation.[17] In the case of mouse spleen cells, concentrations of anti-mouse immunoglobulin antibody which induced cap formation did not stimulate the incorporation of ^{3}H-thymidine.[8]

Some evidence suggests that patch formation is more relevant to B cell triggering than capping. Soluble Concanavellin A (Con A) caps, but does not activate B lymphocytes whereas insoluble Con A does.[1] Phytohaemagglutinin (PHA) and Pokeweed Mitogen (PWM) covalently linked to Sepharose 4B particles, induced a proliferative response in normal spleen cells, similar to that observed with soluble mitogens.[11] With these insoluble mitogens, capping is physically prevented, but patch formation can still occur. When a cell is capped, presumably all the receptors are moved with the cap, then the remainder of the membrane may be in a physical and chemical state not very different from that of the resting membrane, compared to the perturbed state when the receptors are clustered into small patches all over the cell membrane. Thus the biochemical and physiological properties of the capped membrane may be the same as that of the resting membrane, but be altered in the patched membrane.[27]

Activation of lymphocytes by non-specific mitogens, and probably by antigens also, has been described as essentially a cell surface initiated phenomenon.[13] The critical triggering events have yet to be defined, but a series of biochemical changes—(enhanced uptake of ions and metabolites,[12] increased cyclic GMP and increased metabolism of phosphatidylinositol and other phospholipids[14] are said to be initiated by the mitogen induced aggregation of membrane components. Maino *et al.*[20] have presented evidence relating stimulation of enhanced phosphatidylinositol turnover by lymphocyte surface binding ligands to transformation, and supporting the hypothesis that at least in B lymphocytes, it is initiated by cross linkage of the specific membrane components. This investigation demonstrates that the membrane bound immunoglobulin of rabbit peripheral blood lymphocytes undergo redistribution as a result of cross linking by anti-immunoglobulins. A sub-population of these cells are transformed into blast cells, probably mediated via membrane perturbation.

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ලිපිවල සාරාංශ – සිංහල පරිවර්තන

පොල්තෙල් සහ පුන්නක්කුවල ඇති ඇෆ්ලටොක්සින් භායනය කිරීම.

සමරජීව යූ., අර්යකුලරත්න, එස්. එන්. සහ බන්දුනාථ, සී. එච්. එස්. ආර්.

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ඇස්පර්ගිල්ලුස් නම් දිලීර වර්ධනය සඳහා සහ ඇෆ්ලටොක්සින් සමාවයනය සඳහා පොල් මදය ඉතා අගේ ඇති මාධ්‍යයකි. පුස් හටගත් පොල්වලින් ලබාගත් තෙල්වල ඇෆ්ලටොක්සින් අධික ප්‍රමාණයක් ඇත. පිරි සැකසුම් කළ කොප්පරාවලින් වාණිජ පදනමක් මත සාදනු ලබන තෙල්වල පවා අඩු මට්ටමේ හෝ මධ්‍යම ප්‍රමාණයේ ඇෆ්ලටොක්සින් අඩංගු බව පෙනේ. පොල්තෙල් මනුෂ්‍ය ආහාරය සඳහා ගන්නා බැවින් එහි ඇතිවන ඇෆ්ලටොක්සින් දූෂණය මැඩ පැවැත්වීම ඉතා හදිසි කටයුත්තක් බවට පත්වී ඇත.

කලින් සඳහන් කළ ගබඩා කරන ලද පොල්තෙල්වල ඇෆ්ලටොක්සින් ප්‍රමාණය ඉබේම අඩුවී යාම ගැන කරන ලද පරීක්ෂණයකින් හෙළිවී ගියේ සූර්යාලෝකය මගින් ඇතිවන ප්‍රභාභායනය කරණකොටගෙන එසේ සිදුවන බවයි. මෙය සමහර විට සූර්යාලෝකයෙහි ඇති පාරජම්බුල කිරණ නිසා සිදුවන්නට ඇත. වැඩි ප්‍රමාණයක හෝ අඩු ප්‍රමාණයක ඇෆ්ලටොක්සින් සහිත පොල්තෙල්වලට (60 කැල්/සෙ. මී.^{2} ට වැඩි) සූර්යාලෝකය වැටෙන්නට සැලැස්වීමෙන් ඇෆ්ලටොක්සීන් ප්‍රමාණය සැහෙන ලෙස අඩුවූ බව දැනගත හැකිවිය. වෙළෙඳාම සඳහා නිෂ්පාදනය කරන ලද දූෂණය වූ පොල්තෙල්වල ඇෆ්ලටොක්සින් මට්ටම් 30 ppb ප්‍රමාණයටත් වඩා අඩුවන සේ දූෂණහරණය කිරීමට හැකිවිය. එයින් FFA ප්‍රමාණයේ හෝ වර්ණයේ හෝ වෙනසක් ඇති නොවීය.

නිවර්තන කලාපීය රටවල ඇෆ්ලටොක්සීන්වලින් දූෂණය වූ පොල්තෙල් කාර්මික පදනමක් මත දූෂණහරණය කිරීමේ ඉතා කාර්යක්ෂම හා වියදම් අඩු ක්‍රමයක් නිපදවා ගැනීමට සූර්යාලෝක නිරාවරණය යොදගත හැකි යයි මෙම ලිපියෙන් පෙන්වා දී ඇත.

විභාජිත අනුක්‍රමණයක ආධාරයෙන් ඛනිජවල ඇති ආසනික්, ඇන්ටිමනි සහ බිස්මත්වල භූ රසායනය පිළිබඳ තුලනාත්මක අධ්‍යයනයක්

දිසානායක, සී. බී.

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ස්කාර්ගාර්ඩ් ආක්‍රාන්තිය මගින් නිර්දිෂ්ට කොට ඇති පරිදි, පූර්ණ විභාජිත අනුක්‍රමණයක ආධාරයෙන් ඛනිජවල ඇති V වන ගණයට අයත් මූලද්‍රව්‍ය වන ආසනික් (As) ඇන්ටිමනි (Sb) සහ බිස්මත් (Bi) පිළිබඳව කරන ලද තුලනාත්මක අධ්‍යයනයකින් පෙනී ගියේ එම මූලද්‍රව්‍ය වල භූ රසායනික හැසිරීම බොහෝසෙයින්, ඒවාට අයත් රසායනය මගින් පාලනය කරනු ලබන බවය. මැග්මා මිශ්‍රණයෙහි ආසනික්වල As^{5+} බහුල වශයෙන් පවතින්නේය. එම මූලද්‍රව්‍යය, ඛනිජවලදී As^{3+} සහ As^{5+} වශයෙන් ද, සුදුසු ප්‍රමාණය හා ආරෝපණය ඇති අනිකුත් කැටායනවලට ආදේශ වීමෙන්ද යන දෙයාකාරයෙන්ම පවතින්නේය. සංකීර්ණ

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ඔක්සි අයන සාද ගැනීමට ඇති ප්‍රවණතාවය නිසා ආසනික් මූලද්‍රව්‍යයට සිලිකේට්වලදී ජාල සාධකයක් වශයෙන් ක්‍රියා කිරීමට හැකියාවක් ඇත. මුක්ත අයන සෑදෙන විට එම මූලද්‍රව්‍යය ජාල විකාරකයක් වශයෙන් ක්‍රියා කරයි. ආසනික්වල භූ රසායනයෙහි විශේෂ ලක්ෂණය වන්නේ මේ දෙබිඩි හැසිරීමයි. අනික් අතින් බලන විට ඇන්ටිමනිවල 0.05–0.2 දක්වා වෙනස් වන $\frac{Sb^{5+}}{Sb^{3+}}$ අනුපාතයක් ඇති බවත් Sb^{3+} අයනය එහි බහුල වශයෙන් පවතින බවත් පෙනේ. ඇතැම් පෙන්ටවැලන්ට් ඇන්ටිමනිවලින් සංකීර්ණ අයන සෑදී අවශිෂ්ට ද්‍රවයෙහි බහුල බවට පත්වීමට ඉඩ ඇති නමුදු, සමාන අයනීය අරයෙන් යුක්ත වූ ප්‍රධාන මූලද්‍රව්‍ය කැටායනවලට, බොහෝ සෙයින් ආදේශනය වන්නේ Sb^{3+} අයනය බවට සැකයක් නැත. V වන ගණයේ අවසාන මූලද්‍රව්‍යය වන බිස්මත්වල භූ රසායනය සම්පූර්ණයෙන්ම වාගේ Bi^{3+} අයනයෙන් යුක්තවන බැවින් ස්වභාව සිද්ධ ලෙස Bi^{5+} අයනය නොපවතින බව පෙනී යයි. බිස්මත් මූලද්‍රව්‍යය, මැග්නටයිට් සහ ඉල්මනයිට් වැනි ඔක්සයිඩ් ඛනිජ සමඟ එක්වීමට කැමැත්තක් දක්වන නමුදු සිලිකේට් වර්ග සමඟ පැහෙන අවස්ථා විරලය. එය බොහෝ සෙයින් ආදේශනය වන්නේ Fe^{2+} අයනය සඳහාය. මේ අනුව, බිස්මත් ඔක්සයිඩ් ඛනිජ වර්ග ඉතා සුලභව පවතින අතර බිස්මත් සිලිකේට් ඛනිජ වර්ග අත්‍යන්තයෙන්ම විරල වන්නේ ය.

කාබන්හරණ ප්‍රතික්‍රියා සහ ශ්‍රී ලංකාවේ ඉල්ලම්-මිනිරන්වල ප්‍රභවය

හපුආරච්චි, ඩී. ජේ. ඒ. සී.

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මේ ලිපියෙන් සාකච්ඡාවට භාජනය කරනු ලබන ප්‍රශ්නයේ මූලික හරය ඉල්ලම්–මිනිරන්වල ප්‍රභවයට කාබන් මූලාශ්‍රය හේතු වන්නේද යන්නයි. මේ ප්‍රශ්නය ගැන සලකා බැලිය හැක්කේ දිවයිනේ ඇති පාෂාණවල රූපාන්තරණ පසුබිම අනුව පමණකි. චූර්ණමය පරිසරයෙහි කාබන් හරණ ප්‍රතික්‍රියාවලියෙන් ලබාගත් CO_2 පදර්ථය මගින් මිනිරන් ඉල්ලම් සෑදීමට අවශ්‍ය කාබන් ප්‍රමාණය ඇතිවිය හැකි බව පෙනුන අතර, කුස්තුර තල වැනි පාෂාණ ව්‍යුහයන්ට එය ඇතුල් වන්නට ඇතැයි යනුද ඉන්පසුව මේ රටේ පාෂාණ සමඟ ප්‍රතික්‍රියා කිරීමෙන් කාබන් නිෂ්පාදනය වන්නට ඇතැයි යනුද සිද්ධාන්තයක් වශයෙන් ඉදිරිපත් කරනු ලැබේ. ඉල්ලම්–මිනිරන් මතුවිය හැකි භූ විද්‍යාත්මක පරිසරය හඳුනාගැනීමට දැන් අපට හැකිවී තිබීම මේ සිද්ධාන්තයේ ප්‍රායෝගික වටිනාකම වශයෙන් සැලකිය හැක.

චක්‍රඡේද ක්ෂේත්‍රවල ඩයෝපැන්ටනිය සමීකරණ දෙකක්

පොන්නුතුරෙයි, තර්මාම්බිකෙයි

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p, g යන මේවා එකිනෙකට වෙනස් ඔත්තේ ප්‍රමේය මූල වශයෙන් සලකනවා නම් $|\alpha|^2 = p^2$ සහ $|\alpha|^2 = p$ යන ඩයෝපැන්ටනිය සමීකරණවලට $g > \frac{1}{2} p^{p^{2/3}}$ සලකන කල්හි $K = R(e^{2\pi i/g})$ යන චක්‍රඡේද ක්ෂේත්‍රයට අයත් නිඛිලයන් මගින් විසඳුම් නොමැති බව මෙයින් ගෙනහැර දක්වා ඇත. තවද K ට අයත් නිඛිලයන් මගින් සරු විසඳුම් ලබාගත හැකි ඉහත කී දෙවැදෑරුම් සමීකරණවලට අයත් $g = p$ හෝ $g = p^2 + p + 1$ යන මේවාට ගැලපෙන වටිනාකම් හැර තවත් වටිනාකම් p සහ g වලට ඇති බවද ගෙනහැර දක්වනු ලැබේ.

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අනන්ත ප්‍රකාශ මාර්ග පොටෝරියැක්ටරය සහ නැ. මී. 366 දී ආලෝකය විසංගත කිරීම සඳහා පිල්ටරයක්

පෙරෙයිරා, ඩබ්ලිව්. පී. ඩී. සහ කදිර්ගාමනාදන්, පී.

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සාමාන්‍ය භාවිතයේ ඇති ප්‍රකාශ එලක පොටෝරියැක්ටරවල අකාර්යක්ෂම ආලෝක උපයෝජනය, අවශෝෂිත ආලෝක තීව්‍රතාවේ ව්‍යාසනය වැනි අඩුපාඩුකම් රැසක් ඇති බව කවිරුත් දනිති. මේ අඩුපාඩුකම්වලින් වැඩි හරියක් නැතිකර දමන අලුත් රියැක්ටරයක් ගැන, එනම් අනන්ත ප්‍රකාශ මාර්ග පොටෝරියැක්ටරයක් ගැන මේ ලිපියෙන් සාකච්ඡා කරනු ලැබේ. මේ රියැක්ටරයට අදාල සිද්ධාන්තයද ඉදිරිපත් කොට ඇත. නැ. මී. 366 දී ආලෝකය විසංගත කිරීම සඳහා භාවිත කළ හැකි අලුත් රසායනික පිල්ටරයක් වඩා කාර්ය සාධක බවද මෙහිලා විස්තර කොට ඇත.

ඇස්. නිගර් නම් දිලීරයෙන් ලබාගන්නා එන්සයිම් ග්ලූකෝඇමයිලේස්වල ලක්ෂණ කීපයක් සහ රෝපණය, විසංගත කිරීම හා පවිත්‍රීකරණය

ජැන්ස්, ඊ. ආර්., පීරිස්, නිර්මලා, ජයරාජ්, ඊ. ඊ. සහ ද සිල්වා, නිමාලි

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ඇස්පර්ගිල්ලුස් නිගර් නම් දිලීරයෙන් ලබාගත් ග්ලූකෝ ඇමයිලේස් 11 වරක් පිරිසිදු කිරීමෙන් පසුව එහි ලක්ෂණ හා අඩංගු දේ ගැන අධ්‍යයනයක් කරන ලදී. මේ සකස් කිරීම α ඇමයිලේස් සහ අයිග්ලූකෝස්සයිඩේල් රහිතව පැවති බව පෙනුනි. උෂ්ණත්වයේ ප්‍රසාදය පිළිබඳව කළ පරීක්ෂණවලින්, 50°C දී එක්සයිමය නිෂ්ක්‍රියභාවයට පත්වන්නට පටන්ගත් නමුදු වාණිජ මට්ටමකින් පිටි වර්ග ග්ලූකෝස් බවට පරිවර්තනය කිරීම සඳහා එම උෂ්ණත්වය ඉතා උචිත බව හෙළි විය. රෝපණ පෙරනයන්හි α ඇමයිලේස් මට්ටම් පරීක්ෂා කළ හැකි පහසු ක්‍රමයක්ද විස්තර කොට ඇත. DEAE–සෙලුලෝස් ක්‍රමයෙන් කළ පරීක්ෂණවලින් අප්‍රධාන ග්ලූකෝහයිඩ්‍රෝලේස් වර්ගද එහි තිබෙන බව හෙලිවිය.

රට හාවුන්ගේ පර්යන්ත රක්ත වසා සෛට කෙරෙහි ප්‍රති–ඉමියුනෝග්ලොබියුලින් දක්වන ප්‍රසාදය

දිසානායක, එස්.

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රට හාවුන්ගේ පර්යන්ත රක්ත වසා සෛට කෙරෙහි ප්‍රති–ඉමියුනෝග්ලෝබියුලින් මස්තු දක්වන ප්‍රසාදය ගැන මෙහි පරීක්ෂණයට භාජනය කොට ඇත. මතුපිටින් අනාවරණය කොට ගත හැකි ඉමියුනෝග්ලෝබියුලින් වසා සෛට සමඟ එක්වූ විට පරිවේෂ්ටනයට හා හානියට පත්වූ අතර පටල බද්ධ ඉමියුනෝග්ලෝබියුලින් පුනර්වර්ධනයටද පත්වූ බව පෙනුනි. ප්‍රති-ඉමියුනෝග්ලෝබියුලින් උත්තේජනයෙන් පසුව බ්ලාස්ට සෛල බවට පරිණාමනය වූ බවද දක්නට ලැබිණි.

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இந்த இதழின் கட்டுரைகளின் சுருக்கங்கள்

தேங்காய் எண்ணெய், பிண்ணாக்கு ஆகியவற்றிலுள்ள அப்லாடொக்சின்களின் படியிறக்கம்

சமரஜீவா, யூ. அர்சகுலரத்ன, எஸ். என்., பந்துநாத, சீ. எச். எஸ். ஆர்.

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''அஸ்பர்கில்லுஸ்'' என்னும் பங்கசின் (களான்வகை) வளர்ச்சிக்கும் அப்லாடொக்சின் திரட்சிக்கும் மிகச் சாதகமான ஊடகமாகத் தேங்காய்ப் பருப்பு திகழ்கின்றது. பூஞ்சணம்பிடித்த தேங்காய்ப் பருப்பிலிருந்து எடுக்கப்பட்ட எண்ணெய்யில் அப்லாடொக்சின் அதிக அளவில் உண்டு. பதனிடப்பட்ட கொப்பரு மூலம் வர்த்தக முறையில் தயாரிக்கப்பெற்ற தேங்காய் எண்ணெயில் குறைவானதும் நடுத்தரமானதுமான மட்டகளில் அப்லாடொக்சின் அடங்கியுள்ளது. தேங்காய் எண்ணெய் மனித உணவாகப் பயன்படுத்தப்பட்டு வருகின்றபடியால் அதில் ஏற்படும் அப்லாடொக்சின் மாசுபடலானது ஒரு கட்டுப்பாட்டுக்குள் கொண்டுவரப்படல் வேண்டியதாகும். அது உடனடிப் பிரச்சினையாகவுள்ளது.

முன்னர் குறிப்பிட்ட களஞ்சியப்படுத்தப்பட்ட தேங்காய் எண்ணெயில் அப்லாடொக்சின் தானுவே குறைந்து செல்வது பற்றி மேற்கொள்ளப்பட்ட சோதனையொன்றால் ஞாயிற்றின் ஒளி மூலம் ஏற்படும் ஒளிப்படியிறக்கத்தின் காரணமாக அவ்வாறு நிகழுமெனக் கண்டறியப்பட்டுள்ளது. ஒரு வேளை, சூரிய வெளிச்சத்திலுள்ள ஊதாகடந்த ஒளியால் ஏற்படவும் ஏற்படலாம். குறைந்த அல்லது கூடிய அளவில் அப்லாடொக்சின் அடங்கும் தேங்காய் எண்ணெய் மீது (60 கெல் / செ. மீ. 2 வுக்குமேல்) சூரிய வெளிச்சம் படச் செய்வதனால் அப்லாடொகக்சின் அளவு பெரும்பாலும் குறையுமென்பது கண்டறியப்பட்டது. விற்பனைக்காகத் தயாரிக்கப்பெற்ற மாசுபட்ட தேங்காய் எண்ணெயின் அப்லாடொக்சின் மட்டங்கலாவன 30 pp^b. விலிருந்தும் குறையுமளவிற்கு அதனை மாசுபடனீக்கம் செய்யமுடிந்தது. அதன் காரணமாக FFA அளவின் அல்லது நிறத்தின் மாற்றமேதும் ஏற்படவில்லை.

அயன மண்டல வலயத்திலுள்ள நாடுகளில் அப்லாடொக்சின்களால் மாசுபட்ட தேங்காய் எண்ணெய்யைக் கைத்தொழில் அடிப்படையில் மாசுபடனீக்கம் செய்வதற்கான வினைத்திறன் மிக்கதும் செலவு குறைந்ததுமான ஒரு வழியினைக் கண்டுபிடிக்க முன்வருபவர்களுக்குச் சூரிய வெளிச்சத் திறந்த வைப்புமுறை வரப்பிரசாதமாக அமையாலாமென்பது இக்கட்டுரையில் சுட்டிக்காட்டப்பட்டுள்ளது.

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பகுதிப்பாட்டொழுங்கொன்றின் மூலம் கனிசங்களிலுள்ள ஆசனிக்கு, அந்திமனி, பிசுமது ஆகியவற்றின் புவியிரசாயனம் பற்றிய ஒப்பியலாய்வு.

திஸாநாயக்கா சி. பி.

J. Natn. Sci. Coun. Sri Lanka 1977 5 (1):

ஸ்கார்காட்டுத் தலையிட்டு மூலம் எடுத்துக்காட்டப்பட்டுள்ளவாறு, பூரணப்பகுதிப்பாட்டு ஒழுங்கின் துணையோடு கனிசங்களிலுள்ள V ஆம் வகுதிக்குரிய மூலகங்களான ஆசனிக்கு (As), அந்திமனி (Sb), பிசுமது (Bi) ஆகியன தொடர்பில் மேற்கொள்ளப்பட்ட ஒப்பியல் ஆய்வொன்றின்படி அம்மூலகங்களின் புவியிரசாயன நடத்தையானது பெரும்பாலும் அவற்றிற்குரிய இரசாயனத்தைத் தழுவியுள்ளதென்பது புலனாகியது. மாக்மாக் குழம்பினிடத்தில் ஆசனிக்கு மூலகத்தின் As^{5+} பெருந்தொகையான அளவில் உண்டெனத் தெரிகிறது. அம்மூலகமானது கனிசங்களோடு ஒன்றியிருக்கும்போது As^{3+}, As^{5+} ஆகிய வடிவங்களிலும் பொருத்தமான அளவு ஏற்றம் ஆகியன கொண்ட ஏனைய கற்றயன்களுக்குப் பிரதியீடாக அமையப் பெற்றும் இரண்டு நிலைகளில் தோற்றமளிக்கின்றது. சிக்கல் ஒட்சிஅயன்களை உருவாக்கிக்கொள்ளுமியல்புடைய ஆசனிக்கு மூலகம் சிலிக்கேற்றுக் கனிசங்களில் வலைப்பின்னியாக இயங்குகிறது. சுயாதீன அயனுக்கத்தில் அதே மூலகம் வலைபின்னற் திரிபுக்காரணியாகவும் இயங்குகிறது. இந்த இரட்டு நடத்தையானது ஆசனிக்கு சார் புவியிரசாயனத்தின் ஒரு சிறப்புக்கூறாக அமைகின்றது. மற்ற பக்கத்தில், அந்திமனிகளில் 0·05–0·2 வரை வேறுபடுகின்ற $\frac{Sb^{5+}}{Sb^{3+}}$ விகிதமொன்று உண்டென்பதும் Sb^{3+} அயன் கூடுதலாகக் காணப்படுகிறதென்பதும் தெளிவாகிறது. சிலவகையான பென்றுவலன்ற் அந்திமனிகளிலிருந்து சிக்கலான அயன்கள் உற்பத்தியாகி எஞ்சிவிடும் திரவத்தில் கலந்து இருக்கக்கூடியதாயினும் நேரொத்த அயன்களின் ஆரையைக் கொண்ட முக்கிய மூலகக் கற்றயன்களுக்குப் பெரும்பாலும் பிரதியீடாக அமைவது Sb^{3+} அயன்களே. V ஆம் வகுதியின் ஈற்று மூலகமான பிசுமதிக்களின் புவியிரசாயனம் முற்றுகவே Bi^{3+} அயன்களைக் கொண்டிருப்பதனல் இயற்கையாக Bi^{5+} அயன் தென்படுவதில்லை. பிசுகமது மூலகம் மாக்னேட்டு, இல்மனேட்டு ஆகியவற்றைப் போன்ற ஒட்சைட்டுக் கனிசங்களோடு சேருமியபிலைக் கொண்டதாயினும் சிலிக்கேற்றுக்களோடு ஒன்றுவதில்லை எனலாம். அது பெறும்பாலும் Fe^{2+} அயனுக்குப் பிரதியீடாகச் செயல்படுகிறது. இதன்வண்ணம் பிசுமது ஒட்டைசட்டுக் கனிசங்கள் பொதுவாகக் கிடைப்பதாயினும் பிசுமதுச் சிலிக்கேற்றுக் கனிசங்கள் அருமையாகவே கிடைக்கும்.

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காபனகற்றல் தாக்கங்களும் இலங்கையில் காரீயப்படுக்கைகளின் தோற்றமும்.

ஹபுஆராய்ச்சி, டீ. ஜே. ஏ. சி.

J. Natn. Sci. Coun. Sri Lanka 1977 **5** (1):

காபன் வளமே காரீயப்படுக்கைகளின் தோற்றத்திற்குக் காரணம் என்னும் பொருள் பற்றியே இக்கட்டுரையில் ஆராயப்படுகின்றது. இந் நாட்டிலுள்ள பாறைகளின் உருமாற்றப் பின்னணியினைக் கொண்டே இப் பிரச்சினையை ஆராயக்கூடியதாகவுள்ளது. சுண்ணமயச் சூழலின் காபனகற்றல் தாக்கங்களின் மூலம் விடுவிக்கப்பட்ட CO_2 மூலம் காரீயப்படுக்கைகளின் தோற்றத்திற்குப் போதுமான காபன் அளவு உருவாகியிருக்கலாமென்பதோடு, மூட்டுத் தளங்களைப் போல பாறை அமைப்புக்களுக்குள் அது புகுந்திருக்கலாமென்பதும், பின்பு நாட்டுப் பாறைகளோடு ஏற்பட்ட எதிர்த்தாக்கத்தால் காபன் உற்பத்தியானதென்பதும் இங்கு நிரூபிக்கப் பட்டுள்ளது. காரீயப்படுக்கைகள் உள்ள புவிச்சரிதவியல் சூழலைக்கண்டு பிடிக்கும் வசதி இப்பொழுது எமக்குக் கிடைத்துள்ளபடியால் இக் கோட்பாட்டின் நடைமுறை முக்கியத்துவம் புலனாகிறது.

சக்கரத்துமிப் புலங்களிலமையும் இரண்டு தயோபந்தசு சமன்பாடுகள்

பொன்னுத்துரை, தருமாம்பிகை.

J. Natn. Sci. Coun. Sri Lanka 1977 **5** (1):

p,g ஆகியன வெவ்வேறுன ஒற்றை விகிதமுறு முதன்மைகளாகக் கொள்ளுமிடத்து, $|\alpha|2 = P^2, |\alpha|^2 = P$ ஆகிய தயோபந்தசு சமன்பாடுகள் தொடர்பில் $g \} \frac{1}{2} p^{p2/3}$ ஆகக் கொண்டால், $K = R\,(e^{2\pi/g})$ என்னும் சக்கரத்துமிப் புலத்திற்குரிய முழுவெண்களின் மூலம் தீர்வுகள் இல்லை என்பது இக்கட்டுரையால் நிரூபிக்கப்பட்டுள்ளது, மேலும் K வுக்குரிய முழுவெண்களாற் பெறப்படும் திரணமில்லாத் தீர்வுகளுள்ள மேற்படி இரண்டு சமன்பாடுகளுக்குரிய $g = p$ அல்லது $g = p^2 + p + 1$ ஆகியவற்ருேடு இசைந்து வரும் பெறுமானங்களைத் தவிர்ந்த பிற பெறுமானங்களும் p, g ஆகியவற்றுக்கு உண்டு என்பதும் எடுத்தாளப்பட்டுள்ளது.

முடிவிலாக் காட்சிப்பாதை ஒளிவெதிர்த்தாக்கியும் ந. மீ. 336 இல் ஒளிஅலைகளைத் தனியாக்கத் தக்கவொரு வடிகட்டியும்

பெரேரா, டபிள்யு, பீ. டீ., கதிர்காமநாதன், பீ.

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வழமையாக உபயோகிக்கப்பட்டுவரும், காட்சிப்பீட ஒளிவெதிர்த்தாக்கிகள், பெரும்பாலும் திறமைகுன்றிய ஒளிப்பயன்பாடு, உறிஞ்சல் ஒளிச்செறிவினைத் திருத்த வேண்டியமை ஆகிய பல குறைபாடுகள் உடையனவாக விருப்பதை யாவரும் அறிவர். இக் குறைபாடுகளிற் பலவற்றைத் தீர்க்கக் கூடிய புதியதோர் எதிர்த்தாக்கியை அதாவது முடிவிலாக் காட்டிசிப்பாதை ஒளிவெதிர்த்தாக்கியொன்றைப் பற்றி இக்கட்டுரையில் ஆராயப்படுகிறது.

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இவ்வெதிர்த்தாக்கி பற்றிய கோட்பாடு ஒன்றும் எடுத்தாளப்பட்டுள்ளது. ந.மீ. 366 இல் ஒளிஅலைகளைத் தனியாக்குவதற்குப் பயன்படுத்தப்படக் கூடிய புத்தம் புதிய இரசாயன வடிகட்டியொன்று எங்ஙனம் இப்பணிக்கு பேருதவியாய்ச் செயல்படுமென்பதும் விளக்கப்பட்டுள்ளது.

அஸ். நிகர் என்னும் பங்கசுவிடமிருந்து பெறப்பட்ட சில என்சைம் குளுகோ அமைலே சுக்களின் இயற்பண்புகள், வளர்ச்சி, தனியாக்கம், தூய்தாக்கல்.

ஜான்ஸ், ஈ. ஆர்., பீறிஸ், நிர்மலா; ஜயராஜ், ஈ. ஈ., த சில்வா, நிமாலீ ஆகியோர்

J. Natn. Sci. Coun. Sri Lanka 1977 **5** (1):

அஸ்பர்கில்லுஸ் நிகர் என்னும் பங்கசு (காளான்வகை) விடமிருந்து விடுவிக்கப்பட்ட குளுகோஅமைலேசு 11 தடவை தூய்தாக்கப்பட்டு அதன் இயற்பண்புகள், அடங்கும் பொருள்கள் ஆகியவற்றைப் பற்றி ஆராயப் பட்டது. அத் தயாரிப்பில் α அமைலேசு, திரான்ஸ்குளுகோசைடேசு ஆகிய வை இல்லை என்பது தெரிந்தது. வெப்பத்தின் விளைவு பற்றிய சோதனைக ளின் மூலம் 50°C இல் நொதியமானது இயக்கமிலாத் தன்மையை அடையத் தொடங்கியபோதிலும் வர்த்தக அடிப்படையில் பசைமாப்பொருள்களை குளுகோசாக மாற்றுவதற்கு மிகப் பொருத்தமுடைய வெப்பநிலை அது வெனத் தெரிந்தது. வளார்ச்சி வடிகட்டிகளின் α அமைலேசு அளவுகளைப் பரிசோதிக்கக்கூடிய எளிய முறையொன்றும் கண்டுபிடிக்கப்பட்டது. DEAE செலுலோசுமுறைத் தழுவிய ஆய்வுகளின் மூலம் முக்கியமல்லாத குளுகோ ஐதரோலேசுக்களும் அங்கு உண்டெனப்பது கண்டறியப்பட்டது.

முயல்களின் சுற்றயற் குருதி நிணயக்குழியங்களின் மீது இமியுனோகுளோபியுலி னெதிரிகள் காட்டுகின்ற விளைவு.

திஸாநாயக்கா, எஸ்.

J. Natn. Sci. Coun. Sri Lanka 1977 **5** (1):

முயல்களின் சுற்றயற்குருதி நிணயக்குழியங்களின் மீது இமியுனோக்கு ளோபியுலினெதிரிச் சீரங்கள் காட்டுக்கின்ற விளைவினைப் பற்றி இங்கு ஆராயப்பட்டுளது. மேலீடாகக் கண்டுபிடிக்கக்கூடியதாகவிருந்த இமியு னோகுலோபியுலின் நிணயக் குழியங்களோடு ஒன்றியபோது மூடியுருவாகி மாளத் தொடங்கியதோடு மென்சவ்வோடிணைந்த இமியுனோகுளோபியுலின் மறுவளர்ச்சி பெறுதலும் அவதானிக்கப்பட்டது. இமியுனோளோபியுலி னெதிரிகள் தூண்டுதலுக்குட்படுத்தப்பட்டபோது அவை அரும்பிக்கலங் களாக உருமாற்றம் அடைந்தன.

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