described by Shope (7) for papilloma virus in domestic rabbits and pseudorabies virus in cattle, as well as the neuropathic effect produced in mice by high dilutions of certain strains of Newcastle disease virus (8).

VINCENT GROUPÉ
FRANK J. RAUSCHER
*Institute of Microbiology, Rutgers University, New Brunswick, New Jersey*

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7 January 1957

## Biological Concentration by Killer Clams of Cobalt-60 from Radioactive Fallout

After the March 1954 nuclear detonation in the Pacific Ocean, a number of the northern Marshall Islands were contaminated with radioactive fallout (1). Since that time, our laboratory has made periodic surveys of the area to evaluate the residual contamination in plants, marine and land animals, soil, and water (2). Among the specimens collected at two years postdetonation were two "killer" clams (*Tridacna gigas*) that were recovered from the shores of Rongelap Island (3).

The soft tissue of the clams was prepared for analysis by the dry-ash method at 500°C and dissolved in dilute acid. Measurements of gross activity on aliquots of the resulting solution revealed the presence of readily detectable amounts of both beta and gamma radiations. As an aid to identification, the samples were subjected to gamma spectrum analysis in a single-channel analyzer. Gamma photons of energies 1.17 and 1.33 Mev which are identical with those of $Co^{60}$ were observed. Confirmation of the presence of this nuclide was sought by chemical separation and by additional radiation characterization.

Table 1. Cobalt-60 and gross gamma activity in killer clams.

| Specimen | Wet Wt. (g) | Gamma Activity | | $Co^{60}$* (disintegration/min)† |
|---|---|---|---|---|
| | | Gross count/min | $Co^{60}$* (count/min) | |
| A | 1800 | 142,700 | 90,300 | 210,000 |
| B | 882 | 356,700 | 303,000 | 705,000 |

* Corrected for recovery.
† The disintegrations per minute were determined by comparison with a $Co^{60}$ source obtained from the National Bureau of Standards.

To establish the reliability of the analytic procedure, a preliminary experiment was devised for evaluating the exchange of $Co^{60}$ with cobalt carrier and the decontamination efficiency from other radioactive elements. Cobalt-60 tracer and cobalt carrier ($CoCl_2$) were added to a 1-month-old solution of mixed fission products. A control was maintained in which the addition of $Co^{60}$ was omitted. The solution was twice scavenged with ferric hydroxide, using ammonium hydroxide for alkalinization and complexation. The cobalt was then precipitated with α-nitroso-β-naphthol (4). Recovery was determined by the colorimetric nitroso-R salt method (5).

The reliability of the analytic procedure was evident from the results of the preliminary experiment. Cobalt was decontaminated from mixed fission products with 99 percent efficiency, and exchange was complete with a 20-percent yield of both carrier and activity.

This analytic procedure was applied to the specimens. The results of analysis are given in Table 1. For the purpose of comparison, the gross gamma count is also included. The data clearly indicate that the greater fraction of the gamma activity was attributable to $Co^{60}$. In specimens A and B, the activity contributed by this nuclide was 63 and 85 percent of the gross gamma activity, respectively.

To establish the identity of the isolated activity unequivocally, the radiations were characterized by aluminum and beryllium absorption curves and by analysis of the gamma spectra. In each case the characteristics were identical with those of an authentic sample of $Co^{60}$.

The appearance of readily measurable quantities of $Co^{60}$ in the killer clam is noteworthy from two aspects. First, $Co^{60}$ is not a component of fission products. It is therefore assumed that this nuclide was induced from an environmental precursor by the neutron flux accompanying the nuclear detonation. A possible precursor is natural $Co^{59}$, which, when bombarded by neutrons, undergoes the typical (n, γ) reaction to form $Co^{60}$. More importantly, this radioelement was not detected in the numerous fallout-exposed materials analyzed at one year postdetonation (1). Presumably the induced activity was present only in trace amounts. The accumulation of $Co^{60}$ from an environment which for all intents and purposes was infinitely dilute points to the enormous concentrating capacity of the killer clam. Experiments are currently underway to establish whether this property is common to all bivalves.

HERBERT V. WEISS
WILLIAM H. SHIPMAN
*U.S. Naval Radiological Defense Laboratory, San Francisco, California*

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## Observations on a Fast-Moving Protein in Avian Malarial Serum

The alterations occurring in the electrophoretic patterns of the serum proteins of men and animals infected with the malaria parasite have been extensively investigated by the now classical moving-boundary method of Tiselius. The results of these studies have been summarized by Stauber (1) in a recent review of the application of electrophoretic techniques in the field of parasitic diseases. In general, no qualitative changes have been proved, but decreased albumin and increased globulin, particularly alpha-2 and gamma globulin, have been shown to occur. This preliminary report describes a marked qualitative change, which was found by utilizing filter-paper electrophoresis, that occurs in the serum protein patterns of pigeons infected with the 1Pl-1 strain of *Plasmodium relictum* (2)—namely, the appearance of a new component possessing an electrophoretic mobility greater than that of albumin.

Paper electrophoresis offers an important advantage over the Tiselius method because it requires an extremely small sample for analysis. It is thus ideally suited to the serial examination of the blood proteins of small laboratory animals without material interference with the usual course of an induced infection. A modification of the horizontal open-strip method of Grassmann and Hannig (3) was employed in these studies. Four-hundredths of a milliliter of serum was applied by micropipet to Whatman No. 1 filter paper strips (35 by 3.75 cm) immersed in a Veronal-acetate buffer of *p*H 8.6 and ionic strength 0.1. A constant current of 0.5 ma per centimeter of paper width was applied for a period of 19 hours at room temperature (23 to

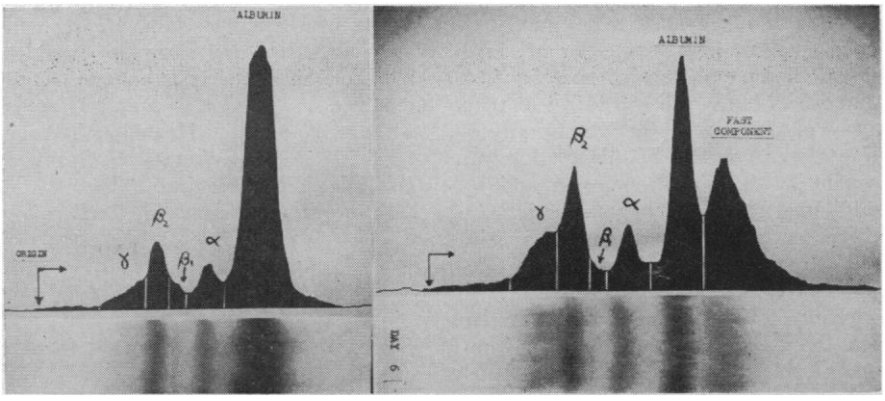


Fig. 1. Comparison between the protein patterns of normal pigeon serum (left) and malarial (right) pigeon serum. The stained strips are shown below the corresponding Analytrol recording.

25°C). Upon completion of the run, the strips were allowed to air-dry for 15 minutes; then they were oven-dried at 70°C for 2 hours and subsequently stained with bromphenol blue for proteins, or with Oil Red O for lipids. Analyses of the stained strips were performed in the Spinco Analytrol, an automatic recording and integrating densitometer.

Figure 1 represents a comparison between the protein patterns of normal and malarial pigeon serum. Normal pigeon serum separates into five distinct bands, whose disposition and intensity are quite similar to the patterns reported for human and other vertebrate serums (*4*). The malarial pattern reveals that gross qualitative and quantitative changes take place as a consequence of the infection. The globulins, particularly gamma globulin, are increased, and the albumin fraction is drastically reduced (*5*). In addition, a new component is evident, whose electrophoretic mobility is greater than that of albumin. It is important to note, however, that this is not the picture of malarial serum per se; it is characteristic of pigeon malaria only during a particular and transient stage in the infection.

This feature was clearly shown when nine pigeons were inoculated with equivalent doses of parasites, and 1 ml of blood withdrawn from each bird daily for a period of 20 days, or until death intervened. A typical series of strips is shown in Fig. 2, where the daily changes occurring in the serum proteins of an infected pigeon may be serially followed. No comparable alterations in the serum proteins were detected in normal birds similarly studied. The fast component emerges early in the infection as a smear on the front of the albumin. It gradually increases in concentration, clearly separates from the albumin, and then progressively disappears, all within the space of a few days.

The superimposed parasitemia curve indicates that the fast component reaches its greatest concentration and maximum separation from albumin after the peak of parasitemia has been passed—that is, during the time that the parasite number is diminishing. It is interesting to note that this is also the period when young red cells are present in the largest numbers in the peripheral blood, as a consequence of the erythropoietic hyperplasia induced by the acute hemolytic anemia so characteristic of malaria in birds (*6*). This fact is highly suggestive of a correlation between stimulated erythropoiesis and the appearance of the fast component in the serum of the pigeon.

Hemolysis of normal pigeon blood by mechanically rupturing the red cells, thus liberating hemoglobin and free nuclei into the serum, did not result in the electrophoretic resolution of a prealbumin component. However, when hemolytic anemia and erythroblastosis were produced in uninfected pigeons by the oral administration of phenylhydrazine hydrochloride, a fast moving protein resembling that described for malaria appeared shortly after initiation of the drug regimen and disappeared soon after cessation of treatment.

The prealbumin fraction of the malarial serum exhibited a blue fluorescence when it was viewed under ultraviolet light, and it stained intensely with Oil Red O, demonstrating that lipid is associated with it. The fast component was isolated by electrophoresis on paper, and its absorption spectrum, as measured in the Beckman spectrophotometer, showed a marked resemblance to that of malarial pigeon albumin, with a maximum at approximately 2800 A and a minimum at approximately 2550 A (*7*).

The findings reported here clearly depict the elaboration of a lipoprotein substance in the serum of pigeons infected with *Plasmodium relictum* whose electrophoretic mobility is greater than that of albumin. The substance seems to be correlated with the erythroblastosis produced by the infection. The similar observations reported for phenylhydrazine poisoning in pigeons would indicate that this phenomenon is a host reaction to pathological disturbances, erythropoietic or hepatic in nature, or both, which may be occasioned either by parasite invasion or drug administration. Additional studies are in progress to characterize the fast component in greater detail, and to clarify further the mechanisms responsible for its elaboration.

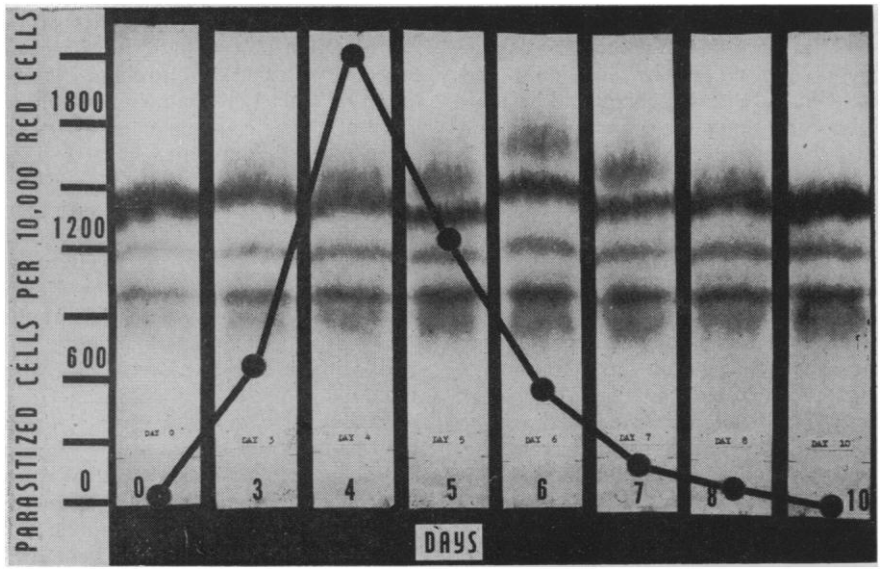


Fig. 2. A typical series of strips showing the daily changes occurring in the serum proteins of an infected pigeon. The preinoculation pattern is shown at the extreme left; the patterns for days 3 through 10 follow. Anodal migration is upward in the figure. The superimposed parasitemia curve represents the intensity of the infection as revealed by counts on daily blood smears stained with Giemsa.

LEWIS A. SCHINAZI*
*Department of Zoology,*
*University of California, Los Angeles*

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* U.S. Public Health Service Predoctoral Research Fellow, National Heart Institute.

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## Concerning the pH Dependence of Enzyme Reactions on Cells, Particulates and in Solution

Considerable attention is being given to the localization of enzymes in and on cells (*1, 2*). Attention has been called to the similarity between the *p*H activity curves of certain enzyme reactions in intact cells and mitochondria (*3*) and in solution. Because of this similarity it has been suggested, for example, with trehalase, lactase, and invertase of yeast, that the enzymes concerned must be peripherally located in the cell. The assumptions are (i) that the internal *p*H of the cell is almost independent of the external *p*H of ambient buffer, (ii) that the permeability of the cell membrane to substrate is independent of *p*H, and (iii) that the extremes of *p*H do not kill the living cells. The assumptions are all valid with yeast (*2, 4*). More recent work with a different approach has verified the conclusion that invertase is located on the surface of yeast cells (*5*).

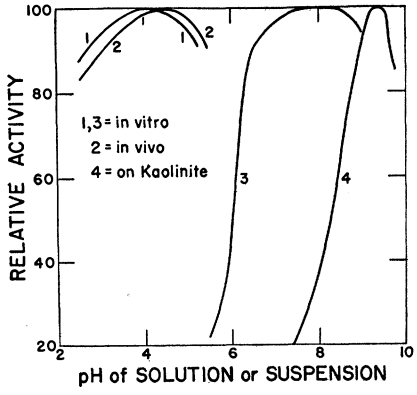


Fig. 1. The effect of *p*H on invertase activity of yeast cells and isolated enzyme (1, 2) and on chymotryptic activity in solution or adsorbed on kaolinite (3, 4).

It is noted here, however, that, if the surface carrying an enzyme is charged, owing to the presence of a polyelectrolyte, one cannot expect that the *p*H for optimum activity of an enzyme will be the same for the enzyme on the surface as compared with the action of the enzyme in solution. A charged surface of a cell or particle will either attract or repel hydrogen ions, depending on the sign of the charge, in an ionic double layer at the surface. An enzyme acting at a surface will thus be exposed to, and in equilibrium with, a hydrogen-ion activity differing from that of the ambient buffer. A comparison of the action of chymotrypsin on a protein in solution and on the surface of kaolinite particles (about 1 μ in size) is a case in point (Fig. 1) (*6*). The *p*H optimum for the enzyme on the surface is at a higher *p*H, and the *p*H of half maximum activity is shifted two units toward higher *p*H, indicating that the hydrogen-ion activity at the surface is about 100 times greater than in solution (*7*). In Fig. 1 data are also plotted from the paper of Wilkes and Palmer (*4*) for the effect of *p*H on invertase activity of yeast cells and of isolated enzyme. Again, the influence of surface is discernible, although less markedly (*8*). As with kaolinite, the data indicate that the surface of yeast has a negative charge density in the *p*H range shown (*9*).


A. D. McLaren

*Department of Soils and Plant Nutrition, University of California, Berkeley*



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24 January 1957


## Enhancement of Oxidative Esterification of Inorganic Phosphate by Clinical Insulin

In an earlier communication (*1*) it was reported that the addition *in vitro* of Lilly clinical plain insulin increased the oxidative esterification of inorganic phosphate by rabbit tissue (kidney and liver) homogenates and extracts. It was subsequently found that similar enhancement could also be obtained with rat kidney and liver homogenates but not with rat brain homogenates. It was further observed that the Wellcome plain clinical insulin also gave similar results. Crystalline insulin (Lilly or Wellcome), however, had no such effect. This suggested that some component of the solvent used in the manufacture of clinical insulin was possibly responsible for the observed effect. Inquiry showed that the solvent now used in the manufacture of clinical insulin contains appreciable amounts (1 to 2 percent) of glycerol. Glycerol is known to act as a phosphate acceptor during aerobic oxidation in rabbit kidney homogenates (*2*). Thus, it appeared that the enhancement of esterification of inorganic phosphate during oxidation by clinical insulin was possibly caused by the presence of glycerol in the insulin.

Table 1 compares the effects of clinical plain insulin (Lilly and Wellcome) and glycerol on aerobic esterification of inorganic phosphate by rat liver homogenates. The results clearly show that the enhancement of the esterification of inorganic phosphate by clinical insulin is caused by the glycerol present in the insulin solution, which acts as an extra phosphate acceptor in the system (*3*). Rat brain tissue possibly does not con-

Table 1. Effects of the additions of clinical plain insulin and glycerol on the oxidative esterification of inorganic phosphate by rat liver homogenates.*

| Experiment No. | Inorganic phosphate esterified (mg) | | |
|---|---|---|---|
| | Control | Insulin | Glycerol |
| 1 | 0.442 | 0.626 | 0.682 |
| 2 | 0.498 | 0.626 | 0.664 |
| 3 | 0.442 | 0.591 | 0.615 |
| 4 | 0.591 | 0.701 | 0.720 |
| 5 | 0.536 | 0.645 | 0.645 |
| 6 | 0.404 | 0.572 | 0.553 |

* Tissue preparation: Overnight-fasted rats were killed by decapitation, and the liver was quickly removed and chilled on cracked ice for 2 to 3 min. The chilled tissue was homogenized in an all-glass homogenizer with 3 vol of isotonic (0.9 percent) KCl for 5 min at 0°C. The homogenate was centrifuged in the cold for 2 min and the supernatant was used in the experiments. Method: Essentially the same as that used in the earlier communication (*1*). Each manometer vessel contained, in the side arm, 0.2 ml of 5 percent glucose and 0.1 ml of 0.2*M* $MgCl_2$, and, in the main compartment, 0.2 ml of 0.2*M* $Na_2HPO_4$, *p*H 7.5, 0.1 ml of 0.5*M* NaF, 0.2 ml of 0.01*M* adenosine-5-phosphate, 0.1 ml of 0.00025*M* cytochrome *c*, 0.2 ml of 0.5*M* sodium succinate, and water to make a final volume of 2.5 ml. When insulin or glycerol was added, 0.05 ml of water in the main compartment was replaced by the same volume of a 40 μ/ml clinical plain insulin (Lilly or Wellcome) or a 1.6 percent glycerol solution in distilled water acidified to *p*H 3.3 by HCl. Duration of experiment, 15 min; gas phase, air; temperature 38°C. Wellcome insulin was used in experiments 1, 2, and 3 and Lilly insulin was used in experiments 4, 5, and 6.