the diluted prothrombin times, which appear inverse to the changes in protamine titers. These return to normal on completion of therapy and can be correlated with the level of adrenal cortical activity by the number of circulating eosinophils (plotted in the lower portion of the figure). Of 20 patients studied, following the first 24 hr of treatment, 12 had an over-all decrease, 6 had no significant change, one had an increase in clotting time, and one had insufficient observations to interpret adequately. Seven of 17 patients with normal initial values had a significant increase in the diluted prothrombin time on therapy. Two had lowered and 5 had elevated protamine

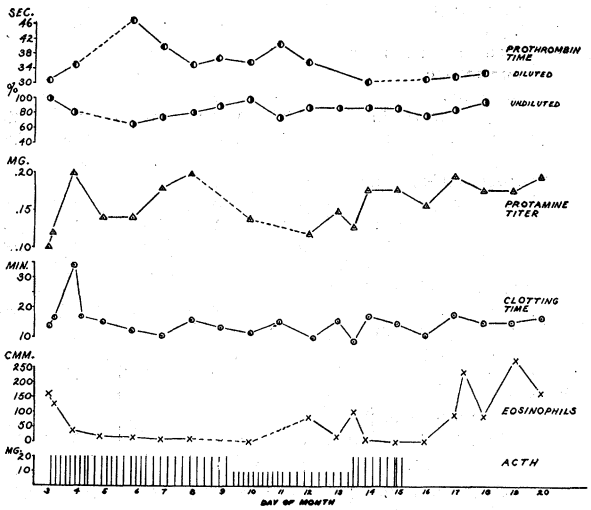

FIG. 4.

titers, of 17 patients in whom this was adequately studied during the period of hormone administration. In addition, there were several patients with high initial protamine titers and low initial eosinophil counts suggesting pre-existing stress, and in these significant changes on treatment were not observed. Platelet counts were not significantly altered.

Figs. 1 and 3 are presented to illustrate the influence of ACTH and cortisone on a number of humoral factors not covered above and known to be active in the coagulation process. It is apparent that variations were produced in the levels of plasma ac-globulin and two-stage prothrombin (Fig. 3) and in ac-globulin and antifibrinolysin (Fig. 1). At present the significance of these changes to blood coagulation in patients receiving ACTH or cortisone is not apparent and will not be further discussed. Similar observations have been made on 6 additional patients under hormonal treatment.

The results of the study herein reported do not allow any broad conclusions, inasmuch as the changes following ACTH and cortisone are by no means uniform from patient to patient. There is little question, however, that the adrenal cortex exerts an effect on a number of constituents of the clotting mechanism and, in some instances, may alter significantly the clotting time when its function is accelerated. The release of heparin or a heparinlike substance into the blood following ACTH or cortisone may be akin to the hyperheparinemia of anaphylactic shock. It affords an additional link in our present understanding of the interrelationship of the mast cell with its heparin production, the adrenal cortex, and the changes in blood coagulability which may accompany the response to stress. The application of the results of the present study to the patient with adrenal insufficiency and to the patient undergoing surgery, among many problems, is obviously indicated and is being currently pursued.

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The Nucleus-Dependence of P^{32} Uptake by the Cell

Daniel Mazia[1] and Henry I. Hirshfield[2]

Department of Zoology,
University of Missouri, Columbia

The various lines of evidence concerning the role of the nucleus in living cells converge in the hypothesis of an ill-defined "determination," or "control," function. The most complete evidence, that from studies of reproduction, heredity, and morphogenesis, is based on experimental designs that give little insight into the problem of the function of the nucleus in the current activities of the mature cell. The fragmentary information on this latter problem, derived from observations on cells deprived of nuclei, has generally been interpreted in terms of two hypotheses (*1*): (1) that the nucleus is a center of essential energetic processes and is immediately involved in the short-term metabolism of the cell, or (2) that the nucleus is concerned only with the long-term maintenance of the cytoplasm. The latter hypothesis derives from observations on cells whose nuclei have been removed. These may survive for some time (a few days to several months [*4*]) in terms of most identifiable activities, but ultimately they decline and die.

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[2] Atomic Energy Commission Postdoctoral Fellow, 1949–50.

For a closer analysis of this problem it is necessary, first, to identify those classes of reactions which are nucleus-dependent and, second, to watch their fate in time following removal of the nucleus. The present experiments deal with the nucleus-dependence of P^{32} uptake in *Amoeba proteus*. This large cell is favorable for removal of the nucleus or for other dissections. It survives for 7–14 days after separation of the nucleus, and the superficial behavior of the enucleated cell has been described thoroughly (*3*). Some data are available on the respiratory rate before and after enucleation (*3*). As will be shown, it is possible to measure the P^{32} uptake of only 30–50 cells.

All the cells used in the experiments were descendants of the same single cell. Large numbers of amebae were first washed completely free of food organisms in phosphate-buffered inorganic culture medium (*2*), the electrolyte composition of which was the same as that of the medium used for stock cultures. Cells were cut, free-hand, into approximately equal halves by glass needles, and the nucleate and enucleate halves separated. Fifty to 100 half-amebae of each class were then transferred into dishes containing 50 ml of medium, to which P^{32} had been added. A similar culture containing the same number of whole amebae was set up. The radioactive medium contained about 10 microcuries of $P^{32}O_4$ per ml. The three cultures were kept at 20° C for 24 hr. The amebae were then washed free from external P^{32} by successive transfers through nonradioactive medium, until samples of the medium showed no measurable traces of radioactivity. Counted numbers of cells were placed in small watch glasses and dried. Their radioactivity was determined by means of a thin-window G-M counter, shielded with 2 in. of lead. Repeated measurements, which included shifting of the sample-dishes and the taking of large numbers of counts, gave reproducible results, and the differences observed cannot be attributed either to statistical fluctuation, although some of the counts were only about 50–100% above background, or to geometry.

The experimental design minimizes certain sources of misinterpretation. The P^{32} uptake was measured in the absence of food organisms and was, therefore, uncomplicated by differences in feeding behavior. In fact, none of the cells divided under these conditions, and the nucleate fragments did not show regenerative growth. Thus, the experiments measure chiefly *basal* processes involving P^{32} uptake. The comparison of nucleate and enucleate halves was undertaken in order to equalize effects of the cutting operation itself. Moreover, the comparison of nucleate halves with whole amebae could give a measure of the extent to which the P^{32} uptake was localized in the nucleus itself.

Since the cells were under microscopic observation throughout the series of transfers, there is no question of the viability of the enucleate half-amebae 24 hr after enucleation, in so far as the term can be applied to a cell without a nucleus. The enucleate fragments tested afterwards showed the behavior pattern characteristic of such individuals, which, in the experience of the authors, survive for 5–10 days before the onset of obvious cytolytic changes.

In considering Table 1, where each column represents an independent experiment, only the experimental values in the same column should be compared. The specific activity of the isotope varied, as did the total P, in the individual experiments. The ratios of the values for the P^{32} uptake by the three classes of cells are comparable for the several experiments. It is seen that the P^{32} uptake by the enucleate fragments is consistently lower, by a factor of 3 or more, than that of the nucleate fragments. This is a real deficit, for the uptake by the nucleate fragments averages only one-half that by the whole amebae, the ratio fluctuating rather widely on either side of 0.5. Therefore, the uptake by the nucleate individuals depends on the amount of cytoplasm, suggesting that the nucleus itself is not taking up a significant proportion of the P entering the cell. This is not surprising. It has been found (*5*) on other types of cells that the rate of P turnover in the nondividing nucleus is considerably lower than the rate in the cytoplasm. In ameba, where the nucleus/cytoplasm ratio is low, the P turnover in the nucleus would have to be very high indeed to make a significant fraction of the turnover by the whole cell.

It is concluded, therefore, that the nucleus plays some decisive part in the P^{32} uptake by the cytoplasm. In the absence of a nucleus, the uptake by the cytoplasm in 24 hr falls to one-third the normal value or less. A simple

TABLE 1

	Test No.				
	1	2*a*	2*b*	2*c*	5
External P (mg/ml)	.037	.10	.10	.10	.10
External P^{32} (counts/min/ml)	2,560	7,168	6,016	2,560	2,690
Background (counts/min)	17	17	22	21	19
P^{32} Uptake (corrected for background)					
Intact amebae					
No. tested	41	50	37	60	75
Total counts/min	59	120	81	50	135
Counts/min/cell	1.2	2.4	2.2	.73	1.8
			(2.6)*	(2.0)*	
Nucleate half-amebae					
No. tested	53	45	34	75	57
Total counts/min	32	81	34	14	55
Counts/min/cell	.65	1.8	1.0	.19	.98
			(1.2)*	(.53)*	
Enucleate half-amebae					
No. tested	80	38	42	87	65
Total counts/min	8	14	13	5	19
Counts/min/cell	0.10	.45	.26	.062	.29
			(.31)*	(.17)*	
Ratios (P^{32} uptake/cell in 24 hr)					
Nucleate half-amebae / Intact amebae	.54	.75	.46	.26	.53
Nucleate half-amebae / Enucleate half-amebae	6.5	4.0	3.8	3.1	3.4

* Corrected for decay.

calculation demonstrates that what is being measured includes incorporation of the P^{32} into organic constituents of the cell. In experiment 2*a*, for instance, the P^{32} concentration of the medium is represented by 7×10^3 counts/min/ml. Taking a value of 50×10^{-4} mm^3 as the volume of a whole ameba, the concentration of P^{32} in the cell is represented by about 2×10^5 counts/min/ml. There is, therefore, more P^{32} in the cell, by a factor of 50–100 for the several experiments, than could be accounted for by complete equilibration with inorganic PO_4. As has been pointed out by others, it is difficult even with data of this type to exclude the possibility that the organic P turnover is limited by the diffusion of inorganic PO_4. However, it has been shown in the case of other cells that the total P uptake is very closely dependent on metabolic variables (*7*), and it has been argued that the initial step in the uptake of PO_4 by the cell is not diffusion across the surface but incorporation into organic compounds (*6*). The conclusion that the present experiments demonstrate a nucleus-dependence of actual P turnover by organic constituents of the cytoplasm is considered probable. Current experiments in which the relative incorporation of P^{32} into various fractions is being determined should provide a more decisive test, as well as closer identification to those processes involving P turnover that are nucleus-dependent.

Although the experiments indicate that metabolic mechanisms in the cytoplasm which can be measured by P^{32} uptake are nucleus-dependent, and that the nucleus itself does not turn over a significant proportion of the P, the question of the nature of the nucleus-dependence remains unanswered. The total P^{32} uptake in 24 hr has been measured. Does the nucleus itself participate directly in the process in some way that does not involve its handling the P, or are we dealing with an indirect, long-term function of the nucleus (*8*)? Examples of the latter would be mechanisms whereby the nucleus was responsible for maintaining the submicroscopic structure of the cytoplasmic systems, or mechanisms whereby the nucleus was essential for the replacement of enzymes that "wore out" during the experimental period. The question may be answered by experiments in which the P^{32} uptake is measured during various intervals after enucleation. If the nucleus participates directly, the effect of enucleation should appear shortly after enucleation. If we are dealing with a replacement mechanism, the effect of enucleation should be more pronounced with time.

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The Use of a Radioactive Isotope in Determining the Retention and Initial Distribution of Airborne Bacteria in the Mouse[1]

L. J. Goldberg and W. R. Leif

Office of Naval Research Task V, Department of Bacteriology, and *U. S. Naval Medical Research Unit No. 1,*[2] *University of California, Berkeley*

In studies of experimental airborne infections, it is important to know the retained dose and the initial distribution of the inhaled material in the host. The classical technique of grinding tissues to determine their bacterial content (*1*) may be an unreliable measure of the viable organisms present. More recently, bacteria that have been radioactively "tagged" have been used in distribution studies following injection (*2*), as have aerosols of inorganic radioactive material following inhalation (*7*). We have employed aerosols of radioactively "tagged" organisms in determining the retention and initial distribution of *Pasteurella pestis* in mice.

A 24-hr culture of an avirulent strain of *P. pestis* was grown in heart infusion broth (Difco) at room temperature on a shaker operating at a rate of 96 oscillations per minute with a stroke of 3 in. Five ml of this culture was transferred to a 100-ml flask of fresh medium containing approximately 1 millicurie of radioactive phosphorus (P^{32}) as soluble phosphate, and incubated as before. The viable count of such a culture was $1–5 \times 10^9$ cells per ml. The organisms were collected by centrifugation (2,000 rpm), washed twice in heart infusion broth, and resuspended in the same medium to yield a concentration of about 1×10^{10} viable cells per ml. In this suspension, one radioactive count per second represented approximately 1×10^5 viable organisms. Additional washings reduced the radioactivity of the cells by uniform amounts (approximately 10%), presumably in consequence of leaching. Two washings were considered sufficient for this investigation.

Mice of the Namru strain (*3*) were exposed to aerosols produced by atomizing suspensions of "tagged" bacteria in the apparatus described by Leif and Krueger (*6*). The average particle diameter in the aerosol was approximately 1 μ, as determined with the particle size analyzer described by Goldberg (*4*).

In the first series of experiments, groups of mice 6–8 weeks old, of both sexes, were exposed to the bacterial aerosol for 20 min. Samples of the aerosol were collected by capillary impingers during the exposure to determine the concentration of radioactive material. Exposed animals were sacrificed within 30 min, skinned, and bisected just below the diaphragm. The thoracic portions

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[2] The opinions and assertions contained in this report are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or of the naval service at large.