SOME ENZYMES OF ISOLATED NUCLEI

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INTRODUCTION

This paper deals with a study of the activities of enzymes in a number of different tissues and in the nuclei isolated from those tissues.

The principal interest in studying the enzymatic composition of nuclei arises from the overwhelming cytogenetic evidence for the importance and uniqueness of their function. Biochemical elucidation of cellular metabolic pathways has usually shown little need to invoke any specific nuclear role, and current studies of cell fractions have yielded results which, except for the already known localization of desoxyribonucleic acid (DNA), but perfunctorily acknowledge nuclear activity. It is an outstanding condition of present interest in the chemistry of nuclei that the persistence in probing their composition derives not from any points of doubt in specific biochemical mechanisms, but from the broad gap of unknowns that intervenes between the well defined genetic data and the sparsely accumulated chemical ones.

In a special sense the field of "biochemical genetics" has dealt chemically with genetic phenomena. More than any other single field of endeavor, it has served to focus attention on the biochemical nature of gene activity, and properly so. Nevertheless, the sweep of this approach, whatever the merits of its one gene–one enzyme proposition and its resultant emphasis on the enzymatic aspect of chromosome behavior, has left untouched, exactly as has the gene-phenotype correlation, that unprobed area of mediation between the hypothetical gene in a chromosome and its observed expression in the organism. Cell physiological studies too, in experiments on nuclear extrusion and in regeneration of an organism such as *Acetabularia*, have on the one hand demonstrated the special importance of the nucleus but on the other, left blank the region in which a knowledge of specific facts alone could point to actual intermediary mechanisms.

One is thus at a stage where the immediate source of orientation is either a generalized picture of nucleoprotein activity as provided by Caspersson and coworkers (1), or a random assembly of enzymatic data gathered from cytochemical procedures of doubtful validity (2). It is for this reason that the present work has been of an exploratory character.

Two aims conditioned the choice of cellular components for study: (*a*) to determine whether nuclei, like cells, were chemically and enzymatically differ-

entiated, and (*b*) to discover some enzymes which might have a specific bearing on nuclear metabolism in general. With respect to the first objective, isolated nuclei were analyzed for those components which characteristically reflect the differentiation of their tissue of origin; arginase of mammalian liver and fowl kidney, uricase and catalase of both liver and kidney, lipase and amylase of the pancreas, alkaline phosphatase of the intestinal mucosa, hemoglobin of the erythrocyte, and myoglobin of the cardiac muscle cell. These are termed "special" components. To explore the general aspect of nuclear metabolism, enzymes of wider distribution were studied. These were the alkaline and nucleotide phosphatases, esterase, β-glucuronidase, adenosine deaminase, guanase, and nucleoside phosphorylase. Selection of these enzymes was conditioned largely by contingencies such as their resistance to inactivation in the course of treatment, ease of their determination, or merely by virtue of their having already been considered by others. With respect to the survival of enzymes in consequence of the non-aqueous method of nuclear isolation (3) limitations were less severe than had at first been anticipated. It has been our experience that, generally, enzymes which can be prepared from acetone powders are active under the conditions of these studies. The field of choice is thus sufficiently broad.

With progress of investigation certain features became clear: there was no typically nuclear enzymatic pattern, and consequently nuclei from a maximum number of diverse tissues had to be analyzed with a view to understanding the relations between different nuclei and the differentiation of their respective cells. Furthermore, if nuclei varied from tissue to tissue, the possibility that the nuclear composition might fluctuate with the physiological state of the cell had to be considered. For this reason the liver of a fasted animal was studied.

The main results of this investigation indicate, first, that nuclei are differentiated, and, secondly, that enzymes related to nucleoside metabolism occur in high concentration in the nucleus. It has also been possible to demonstrate that the relative enzymatic activities of nuclei change markedly with corresponding changes in physiological state of the cell.

Methods

In the absence of methods for determining the actual enzymatic content of a tissue or nuclear preparation, the most useful procedure was to measure *activities* expressed in terms of unit dry weight. In this, of course, there is no assurance that the inactive zymogen form of an enzyme may not be present; one can only presume that there is a reasonable correspondence between *in vitro* activity and the situation obtaining *in situ*. This problem does not arise in experiments with hemoglobin and myoglobin, for in these cases the actual content per unit dry weight is determined.

As routine, assays were first made on suspensions and extracts of these.

Where no appreciable difference in activities was found, convenience dictated the choice of procedure; where the use of suspensions alone is indicated, it is to be understood that extracts were insufficiently active. Suspensions were prepared by weighing the desired amount of dry tissue in a test tube adapted to fit a hemispherical ground glass pestle, and homogenizing it at 0°C. in a known volume of 0.9 per cent NaCl (unless otherwise specified). Extracts were obtained by centrifugation of the homogenate. Where spectrophotometric methods of analysis were used, extinctions were measured in the Beckman spectrometer using cuvettes of 1 cm. light path.

Amylase.—

Homogenization of tissue or nuclei was carried out in a solution of 0.02 M NaCl and 0.01 M phosphate buffer (pH 7.1). Suspensions and extracts were equally active. The activity measurement is based on the colorimetric determination of liberated maltose (by its reaction with dinitrosalicylic acid) when amylase acts on a soluble starch substrate. The procedure followed was that of Meyer *et al.* (4) with this modification: 2 ml. samples were periodically removed from the incubation mixture and transferred to tubes containing 0.5 ml. of 20 per cent trichloracetic acid. To 1 ml of the supernatant obtained by centrifugation, 1 drop of brom thymol blue was added and the whole titrated with 0.3 N NaOH to a green color. The solution was made up to 2 ml. with water and 1 ml. of the 3,5-dinitrosalicylic acid reagent added. Extinctions were read at 530 mμ.

Activity: mg. maltose liberated /mg. tissue /30 minutes at 37.5°C.

Arginase.—

Suspensions were assayed by the procedure of Van Slyke and Archibald (5), which is based on Archibald's method (6) for the colorimetric determination of the urea liberated when the enzyme acts on arginine solutions.

Activity: mM urea liberated /mg. tissue /20 minutes at 30°C.

Catalase.—

Extracts and suspensions were equally active. The method followed was that of Euler and Josephson (7) based on permanganate titration of hydrogen peroxide before and after incubation with the enzyme.

Activity: μM H_2O_2 liberated /2.5 mg. tissue /3 minutes at 0°C.

The possibility of DNA inhibiting catalase activity in suspensions of nuclei was tested by studying inhibition of tissue suspensions. When appropriate amounts of DNA were added to suspensions of whole tissue, no inhibitory effect was observed.

Esterase and Lipase.—

Suspensions were used since extracts were only one-third as active. The colorimetric method developed by Seligman and coworkers (8, 9), using β-naphthyl laurate as substrate for esterase and β-naphthyl palmitate-stearate as substrate for lipase, was exactly followed. This method depends on the colorimetric determination of the β-naphthol released by enzymic hydrolysis of the substrates mentioned. The naphthol

is determined by coupling with a diazotate, followed by extraction of the resulting pigment in ethyl acetate. In line with the observation of Seligman *et al.*, activities classified as due to esterase were confirmed by the inhibitory effect of sodium taurocholate (inhibition was about 50 per cent in whole tissues and in nuclei), and those considered as due to lipase, by the activating effect of sodium taurocholzte. In the final step of the procedure the pigment was always extracted in 3 ml. of ethyl acetate and its extinction measured at 530 mμ.

Activity: (*a*) Esterase— E /mg. tissue / 10 minutes at 27.5°C. (except for calf thymus which was incubated at 37.5, and for beef pancreas which was measured at 0°C.

(*b*) Lipase— E /mg.tissue / 10 minutes at 15°C.

β-Glucuronidase.—

12 to 20 mg. of tissue was homogenized in 1.4 ml. of saline and made up to 7 ml. with 0.1 M acetate buffer at pH 4.5. The activity measurement is based on the colorimetric determination of the phenolphthalein released when the enzyme acts on phenolphthalein glucuronide. Proportionate amounts of the various components of the reaction mixture described by Rossiter and Wong (10) were added to the suspension and the whole incubated at 37.5°C. Periodically, 1 ml. samples were pipetted into tubes containing 0.3 ml. of a 20 per cent sodium tungstate solution. The free phenolphthalein was extracted with ether which was then transferred to a clean tube and evaporated to dryness. The residue was redissolved in 1 ml. of 0.1 M glycine buffer (pH 10.9) and its extinction at 553 mμ read immediately.

Activity: E /mg.tissue / 60 minutes at 37.5°C.

Nucleoside Phosphorylase, Adenosine Deaminase, and Guanase.—

Tissues were extracted for 2 hours in the cold with 0.2 M phosphate buffer (pH 7.4), and the clear extracts used for all determinations according to the spectrophotometric procedures outlined by Kalckar (11), based on the characteristic changes in ultraviolet absorption accompanying the enzymic conversion of substrate during the reaction. For nucleoside phosphorylase the hypoxanthine produced from inosine (or desoxyinosine) was converted to uric acid by adding an excess of xanthine oxidase and the increase in absorption at 290 mμ measured. The deamination of adenosine to inosine was followed by the decrease in absorption at 265 mμ. The xanthine produced by the deamination of guanine was determined by the same procedure used for hypoxanthine. In the case of nucleoside phosphorylase, it was found that in nuclei and whole tissue of kidney, liver, and thymus, the desoxyriboside was consistently attacked at about half the rate measured for the riboside; the remainder of the tissues were therefore tested with inosine alone. Since tissue uricase, if active under the conditions, would interfere with the determinations of nucleoside phosphorylase and guanase by destroying the end-product of both reactions, the possibility of its interference was tested in the following ways using horse liver, which has a high uricase activity, as source of extract:

1. An appropriate amount of hypoxanthine was added to the liver extract in the presence of xanthine oxidase. After incubating for 10 minutes at 37.5°, the ΔE_{290} was determined and found to account quantitatively for the hypoxanthine added.

The cuvettes were then returned to the water bath for an additional 30 minutes and the extinction at 290 mμ again read. No significant decrease was observed. Therefore, no uric acid was decomposed under these conditions.

2. Uric acid was added to the liver extract and the ΔE_{290} was recorded after 10/ minutes of incubation. No change was observed.

The initial concentration of substrates (inosine, adenosine, guanine) was approximately 12 γ / ml., and the amount of extract used was adjusted so that no more than 2 to 4 γ of substrate was decomposed in the first 10 minutes. Readings were also taken after 20 minutes to assure that the rate of reaction was approximately linear.

Activities: (*a*) Nucleoside phosphorylase— γ hypoxanthine liberated per mg. tissue /10 minutes at 37.5°C.

(*b*) Adenosine deaminase— γ adenine deaminated per mg. tissue /10 minutes at 37.5°C.

(*c*) Guanase— γ guanine deaminated / mg.tissue /10 minutes at 37.5°C.

Phosphatases.—

(*a*) *Alkaline Phosphatase.*—Suspensions of tissue were buffered with carbonate to a pH of 9.7 in the presence of 0.002 M $MgCl_2$ and incubated with phenolphthalein diphosphate as described by Huggins and Talalay (12). A sample of the monophosphate (generously given to us by Dr. A. Gutman) was also tried but much the same pattern of results was obtained. Generally 5 to 10 ml. of incubation mixture was used and 1 to 2 ml. samples were periodically removed and analyzed for phenolphthalein as in the procedure for β-glucuronidase. Under these conditions the amount of phenolphthalein liberated, in contrast to the findings of Huggins and Talalay for serum phosphatase, was proportional to the period of incubation and to the concentration of tissue.

Activity: γ phenolphthalein liberated /mg.tissue /hour at 37°C.

(*b*) *Adenosinetriphosphatase.*— Suspensions were treated in the manner described by DuBois and Potter (13). The amount of liberated phosphate was, however, more conveniently determined by the method of Berenblum and Chain (14).

Activity: γ P liberated /mg.tissue /hour at 37.5°C.

(*c*) *Adenosine-3-* and *-5-Phosphatases.*— Suspensions were first dialyzed overnight against distilled water as this treatment increased activity. The reaction mixture containing either adenosine-3 or adenosine-5-phosphate was buffered to a pH of 8.9 with carbonate; the remainder of the procedure, *i.e.* the determination of liberated inorganic phosphate, was the same as for the ATPase determination.

Uricase.—

Tissues were extracted with 0.1 M glycine buffer (pH 9.5) and the extracts incubated with uric acid. The decrease in extinction at 292 mμ was a measure of the decomposition of the uric acid.

Activity: E_{292} /mg.tissue /hour at 37.5°C.

Total Iron and Heme Iron.—

Total iron was determined colorimetrically as the ferrous phenanthroline complex according to the method of Sandell (15) except for the use of *p*-nitrophenol as indi-

cator in neutralizing the acid digest. Heme iron was obtained by subtracting *non-heme* iron from the total iron content. The procedure of Bruckmann and Zondek (16) was slightly modified for determining non-heme iron. Enough material to yield 3 to 10 γ of non-heme iron was homogenized in 2 ml. of a 2:1 mixture (by volume) of 10 per cent trichloracetic acid and saturated sodium pyrophosphate. The suspension was shaken for 18 hours at room temperature and then centrifuged. The supernate plus two washings with a 1:1 mixture of the same reagents was collected in a tube and heated in a salt bath to convert the pyrophosphate (which interferes with the hydroquinone reduction of the iron) to orthophosphate. The remainder of the procedure was the same as for total iron determination.

The bulk, if not all, of the heme iron of erythrocyte nuclei is in the form of hemoglobin. This was shown by preparing crystalline hemoglobin in high yield from the isolated nuclei.

Tabulations.— "C" and "N" are used as abbreviations for cytoplasm and nucleus, respectively. The values for cytoplasmic activity have been calculated from those experimentally determined for the whole tissue and isolated nuclei. The known fractional weights of the nuclei (3) make this calculation possible. Where nuclear activity is below 7 per cent of that determined in the cytoplasm, such activity cannot be considered as conclusively demonstrated in view of the possible error of cytoplasmic contamination of the nuclear preparation.

RESULTS

(*A*) *The Nuclear Content of "Special" Components and Generally Distributed Enzymes in Adult Tissues*

In comparing the compositions of nuclei prepared from various organs the one general and most impressive feature is their characteristic difference. Hemoglobin, for example, is present in the erythrocyte nucleus but in no other; only liver nuclei contain catalase although the enzyme also occurs in the kidney; nuclear arginase is found exclusively in liver and in calf kidney, not in fowl kidney; and guanase, which occurs in liver nuclei of the horse and rat, is not detectable in calf liver nuclei. For enzymes of general distribution, *e.g.* nucleoside phosphorylase, the variations in activity between nuclei of different tissues are as great, if not greater than those between the tissues themselves. The results are thus unequivocal on this point: Nuclei show little uniformity of enzymatic pattern and are as variable as their sources of origin. In the sense that differentiation is the result of a developmental process tending to the formation of characteristic differences, nuclei may be considered to be differentiated structures.

1. "Special" Enzymes.—

The nuclear concentration of several substances which characterize the tissues in which they occur is given in Table I.

Although much of the evidence adduced for nuclear differentiation rests

upon the intracellular distribution of these so called "special" enzymes, no simple relation is to be found between the occurrence of these enzymes in the tissues and their activity in the corresponding nuclei. Lipase, amylase, uricase, and alkaline phosphatase are, in adult tissues at least, either absent from nuclei or present in very low concentrations. Arginase and catalase, on the other hand, are, in certain cases, present in both nucleus and cytoplasm. No apparent basis was found to explain the difference in behavior between the two groups of

TABLE I

Intracellular Distribution of "Special" Enzymes

	(*a*) Arginase				
	Liver			Kidney	
	Calf	Horse	Fowl	Fowl	Calf
C	2.88	1.21	0.04	0.620	0.137
N	1.55	0.6	0.025	0.040	0.152
100 N/C	54	57	63	7	110

	(*b*) Catalase				(*c*) Uricase		
	Liver		Kidney		Liver		Calf Kidney
	Horse	Calf	Fowl	Calf	Calf	Horse	
C	41	9.3	33.5	25	0.41	2.14	2.9
N	29	3.0	0	0	0	0.11	0
100 N/C	71	32	0	0	0	5	0

	(*d*) Pancreas				(*e*) Intestinal mucosa (calf)	
	Lipase		Amylase		Alkaline phosphatase	Adenosine deaminase
	Horse	Beef	Horse	Beef		
C	0.323	0.655	14.30	36.05	344	127
N	0.005	0.055	0.356	2.57	12	24
100 N/C	1.5	8	2.5	7.1	3.5	19

enzymes. Physical factors, such as differences in solubility or molecular size, do not explain the observed intracellular distribution. Both lipase and amylase, for example, appear to be absent from pancreatic nuclei although amylase is completely soluble in saline whereas lipase is only slightly so. Catalase and arginase, on the other hand, occur in some nuclei, yet catalase is completely soluble while arginase (though completely extractable from liver nuclei) is only partly extractable from the whole tissues of liver and kidney. This lack of correlation between solubility and nuclear activity extends to enzymes of wider distribution. For example, a striking contrast is provided by the closely related enzymes, lipase and esterase, both of which are incompletely soluble in saline;

lipase activity is hardly detectable in isolated nuclei whereas esterase activity is frequently present in nuclei to a high degree.

The reason why arginase should be present in calf liver nuclei and absent from those of fowl kidney, or why catalase, which is active in the tissues of both liver and kidney, should be restricted to nuclei of liver alone, is not clear at present. It would appear that inferences from data confined to tissues at the adult level are bound to be inadequate, and that a better understanding of the relation between nucleus and cytoplasm could be achieved by examining the intracellular distribution of these special enzymes at various stages in the ontogeny of the tissues concerned.

TABLE II

Hemoglobin and Myoglobin in Erythrocyte and Heart Tissue

	Goose		Fowl		Beef (heart)	
	Cells	Nuclei	Cells	Nuclei	Cells	Nuclei
Total Fe, *per cent*	0.21	0.08	0.258	0.104	0.0267	0.0098
Nonheme Fe, *per cent*	0.06	0.034	0.0525	0.0353	0.0125	0.010
Nuclear hemo- or myoglobin, *per cent*	14		19		0	
100 N/C (heme Fe)	33		34		0	

2. Hemoglobin and *Myoglobin.*—

The results of total and non-heme iron analyses of avian erythrocyte and cardiac muscle nuclei are summarized in Table II.

In contrast to measurements of enzymatic *activities*, the study of hemoglobin and myoglobin has two distinct advantages: First, the absolute concentration of either of these substances can be accurately determined by iron analyses, as described above under Methods, and secondly, concentrations so determined are independent of a number of inactivation and inhibition problems which beset enzyme activity determinations. Thus, there is the possibility that in the course of preparing the nuclei, a partial inactivation of some of the enzymes studied might occur. For this reason, a "tissue control" was prepared from all tissues by treatment of the ground suspension with the same solvents for the same time as the isolated nuclei (3). Nevertheless, one could suppose that somehow the enzymes in the nuclei were more sensitive to the treatment than those in the tissue, or *vice versa*, and that the destruction was, therfore, selective. Even if this unlikely possibility were to occur, measurements of hemoglobin and myoglobin concentrations would be entirely unaffected.

The intracellular distribution of these pigments shows much the same complex pattern as that observed for the "special" enzymes. On the one hand, 19

per cent of the dry weight of fowl erythrocyte nuclei can be accounted for as hemoglobin. If one considers that the nucleohistone fraction is approximately 64 per cent of the erythrocyte nucleus, it follows that more than half of the remaining protein is hemoglobin. Yet, in contrast to this impressive concentration in the fowl erythrocyte nucleus, the myoglobin of beef heart is entirely confined to the cytoplasm. Again no simple explanation is possible. If diffusibility were a factor, myoglobin with a molecular weight only one-third that of

TABLE III

Intracellular Distribution of Phosphatases

		Calf					Horse liver
		Intestinal mucosa	Thymus	Kidney	Heart	Liver	
Alkaline phenolphthalein phosphatase	C	344	37	20.4	—	4.32	17.4
	N	12	0.17	1.3	—	0.5	4.9
	100 N/C	3.5	0.5	6	—	12	28
ATPase	C	63	9.13	31	9	39.5	
	N	0	0.86	0	0	4.7	
	100 N/C	0	9	0	0	12	
Adenylic-5-phosphatase	C	50	7.33	—	<0.2	17.2	
	N	3	0.66	—	0	3.1	
	100 N/C	6	9	—	0	18	
Adenylic-3-phosphatase	C	41	4.75	—	<0.2	4.4	
	N	1.7	0.51	—	0	0.45	
	100 N/C	4	11	—	0	10	

hemoglobin, would be expected to be in the nucleus, but the opposite is the case.

Nucleus and cytoplasm of avian red cells also differ in their ratios of nonheme to total iron, this ratio being 29 per cent in the intact goose erythrocyte, and 43 per cent in the nucleus.

3. Enzymes of General Distribution.—

(*a*) *Phosphatases.*—The intracellular distribution of alkaline and nucleotide phosphatases in different tissues is summarized in Table III.

The localization of these enzymes has been a popular subject of investigation. Frequently much importance has been attached to their role in the metabolism of nuclei (2, 17), although a large part of the evidence was adduced from histochemical procedures of doubtful validity (18). Alkaline phosphatase activity, in particular, has occupied a central role in these studies despite the undue com-

plexity of its *in vitro* behavior. In a comprehensive review of the properties of this enzyme, Roche and van Thoai (19) have pointed to the absence of uniform conditions for its activation in homogenates of different tissues, so that no single procedure is available for assuring maximum activity in comparative assays of different organs. In reporting the results of its distribution we therefore emphasize that the significance of the data may be limited by the fact that all our activity measurements were made under identical, but not necessarily optimal, conditions. Nevertheless, in intestinal mucosa, in which the extremely high alkaline phosphatase concentration permits its classification as a "special" enzyme, there can be little doubt that its presence in the nucleus is of minor proportion. In fact, attempts were made to increase the nuclear activity by autolyzing nuclear suspensions (in conformity with the procedure used in purifying the enzyme) or by adding various divalent ions, but in no case were differences sufficient to alter the evidence obtained for its almost complete absence. In other tissues, too, in which alkaline phosphatase is not in the category of a special enzyme, nuclear activities were only occasionally beyond the possible errors of contamination. (Horse liver nuclei were an exception.)

The nucleotide phosphatases showed much the same pattern of distribution; *i.e.*, generally low nuclear concentration. It is of some interest that although the 5-phosphate linkage has been demonstrated for the phosphate-sugar bond in DNA, the enzyme highly specific for its hydrolysis (adenosine-5-phosphatase) should be so poorly represented in the nucleus.

(*b*) *Esterase.*—As indicated in Table IV, this enzyme is variably active in nuclei of different tissues. Generally, the nuclear concentration is appreciable, except in intestinal mucosa and the medullar portion of the kidney. The reason for the low activity in the latter case is not at all apparent, but the exceptional value of mucosa nuclei can be fitted into a reasonable scheme which will be discussed in connection with fetal tissues.

(*c*) *β-Glucuronidase.*—The intracellular distribution of this enzyme is also summarized in Table IV. Special interest in β-glucuronidase arises out of its presumed association with proliferative activity. The nature of the relation is still obscure and some pertinent evidence accumulated in this laboratory will be presented separately. Of immediate significance, however, is the fact that β-glucuronidase is not concentrated in nuclei, with the possible exception of those of the mucosa.

(*d*) *Nucleoside-Specific Enzymes.*—The intracellular distribution of adenosine deaminase, nucleoside phosphorylase, and guanase in different tissues is listed in Table IV.

It will be recalled that uricase and the nucleotide phosphatases, enzymes catalyzing the catabolic reactions of purine compounds, are poorly represented in nuclei of all tissues. It is a striking contrast that enzymes concerned with the metabolism of the nucleosides are concentrated in most nuclei. One of these

enzymes, nucleoside phosphorylase, is quite possibly concerned with nucleoside synthesis, but the supposition of synthetic functions for adenosine deaminase and guanase is questionable.

It is clear from the data of Table IV that both adenosine deaminase and guanase cannot be considered essential to a universal pattern of nuclear metabolism, since neither of these enzymes is present in all nuclei. In calf kidney, both tissue and isolated nuclei show only a trace of adenosine deaminase ac-

TABLE IV

Intracellular Distribution of Commonly Occurring Enzymes in Adult Tissues

		Calf							Horse liver
		Liver	Kidney	Kidney cortex	Thymus	Heart	Intestinal mucosa	Pancreas	
Esterase	C	0.161	0.061	0.078	0.075	0.011	0.336	0.398	0.160
	N	0.122	0.006	0.018	0.026	0.014	0.029	0.128	0.128
	100 N/C	76	10	23	35	127	9	32	80
β-Glucuronidase	C	1.88	0.50		0.655	T r a c e	0.410	T r a c e	0.433
	N	0.324	0.06		0.033		0.102		0.073
	100 N/C	17	12		5		25		17
									Guanase
Adenosine deaminase	C	52	T r a c e	T r a c e	80	7	127	0.81	4
	N	108			53	42	24	1.32	5.5
	100 N/C	208			66	600	19	163	138
Nucleoside phosphorylase	C	52	15	17	19	18.5	19	28	15
	N	52	19	17	7	82	4.5	21	41
	100 N/C	100	120	100	37	440	24	75	274

tivity. In the livers of the calf, horse, and rat, adenosine deaminase and guanase would appear to be mutually exclusive enzymes; for, in calf liver only adenosine deaminase is present, and in the livers of the horse and rat, only guanase. Unfortunately, normal rat liver nuclei are difficult to prepare in pure condition and were not used as routine in enzyme analyses. A morphologically unsatisfactory preparation was tested, however, for nucleoside phosphorylase and guanase activity; the values obtained were sufficient to indicate that the intracellular distribution of these enzymes was essentially similar to that observed in horse liver.

Nucleoside phosphorylase is the most widely distributed of the three enzymes, its activity being appreciable in all tissues examined. Its function is

also of special interest. The supposed specificity of this enzyme for ribosides and desoxyribosides of guanine and hypoxanthine (20) has now been extended by Rowen and Kornberg (21) who have indicated its capacity to catalyze the formation of nicotinamide riboside from nicotinamide and ribose-*l*-phosphate, a step in the synthesis of diphosphopyridine nucleotide. It cannot be inferred that such activity is the basis for the concentration of this enzyme in nuclei, but the characteristic intracellular distribution does provide sufficient ground to speculate that nucleoside phosphorylase is of special significance to the metabolism of most nuclei.

It will be noted that intestinal mucosa nuclei have an exceptionally low concentration of the nucleoside-specific enzymes. A tentative explanation of this is offered in connection with data on fetal tissues. A further point of interest is the sharp contrast in relative nuclear activity (N/C) between thymus and heart nuclei, for the latter are the most active of all nuclei tested, and thymus nuclei are only slightly active. It is possible that this difference in activity is inversely related to the nuclear fraction of the cell, because thymus nuclei constitute 60 per cent of the dry weight of their cells, and heart nuclei comprise only 5 per cent of the tissue. Between these two extremes, however, there is no clear correlation between relative nuclear activity and relative nuclear size.

(*B*) *Physiological State and Enzyme Activity*

The net result of the above studies has been to show that nuclei are enzymatically differentiated and that some enzymes are consistently found in most cell nuclei in relatively high concentrations. It cannot be assumed, however, in view of the profound influence which the nucleus is known to have on the history of the cell, that the patterns of distribution found in adult tissues are of a fixed character. For this reason the possibility of fluctuations in nuclear activity occurring with changes in physiological state was investigated.

1. Fasting.—

Within the range of our present techniques the simplest method of examining the proposition of nuclear variability was to study some organ under conditions of starvation. For a tissue such as liver the change of state is real and radical; notably there is a shift from carbohydrate to protein metabolism, the latter utilizing the protein stores of the tissue itself. Accordingly, an experiment was performed in which a horse weighing 1270 pounds was fasted for 20 days, his original weight being reduced by 20 per cent. The liver was then removed and the nuclei were isolated by the procedures described in the preceding paper (3).[1] The enzymatic composition of these "fasted" nuclei was then compared with the measured activities of normal liver nuclei.

[1] The authors are greatly indebted to Mr. Oxfeld and associates at the Eastern Packing Co., Newark, New Jersey, whose cooperation made this experiment possible.

In considering the comparative data on fasted and control livers, the most useful point of departure is the demonstration by Mirsky and Kurnick (unpublished) that in both rats and rabbits the DNA content of the cell remains constant during starvation. It may be safely assumed that this constancy is also the rule in the present experiment, and that relative enzymatic activities may therefore be calculated on a *per cell*, rather than on a dry weight basis. The usefulness of such consideration arises from the fact that starvation produces an appreciable drop in the protein content of both the cell and the nucleus; in horse liver this decrease amounted to 28 per cent in the cell, and 33 per cent in the nucleus. It follows that the computation of enzymatic activities on a dry weight basis leads to high results in fasted tissue because more "fasted" cells are represented in a given weight of sample. To avoid this difficulty the data have been corrected in the following way: The enzyme activity measured in the fasted tissue was multiplied by the factor 105/146, the ratio of DNA-phosphorus in normal liver to that of fasted liver. Similarly, nuclear activities after fasting were multiplied by the factor 120/178. This correction makes it possible to evaluate the change in enzymatic activity on a cellular or nuclear level.

The effect of fasting on the intracellular distribution of seven enzymes is summarized in Table V. Only catalase activity is decreased; the remainder of the enzymes show a variable but definite increase. (If the above correction had not been applied, the apparent increase would have been much greater.) What is interesting in this change, apart from its relation to adaptive enzyme behavior, is that the activity changes in the nucleus proceed in the opposite direction to those observed in the tissue. There is not only an absolute drop in enzymatic activity per nucleus, but an even greater drop in the nuclear/cytoplasmic activity ratio. Such behavior of nuclei is in strong contrast to the constancy in DNA content and clearly establishes the fact that in the somatic cell the enzymatic activity of nuclei varies both absolutely and relative to the cytoplasm with a change in physiological state. It takes little extrapolation to appreciate the limited aspect of the data presented in section A. Obviously, the degree of enzymatic activity in a nucleus is related not only to its tissue of origin, but also to the physiological state of that tissue at the time of its excision.

2. Comparison of Fetal with Adult Tissues.—

Since nuclear composition varies in the adult state one might guess that even greater variations occur in the course of development. The study of these developmental changes in the nucleus is severely limited by present techniques. The minimum amount of tissue necessary for isolation of nuclei requires a large number of fetal organs. The collection of such numbers for a study of specific fetal stages was a practical impossibility, so that comparisons with adult tissues had to be obtained for pooled organs in various stages of development. The results, nevertheless, are of sufficient interest to justify the procedure used.

TABLE V

Effect of Fasting on the Intracellular Activity of Horse Liver Enzymes

	Nucleoside phosphorylase		Esterase		Arginase		β-Glucuronidase		Uricase		Catalase		Alkaline phosphatase	
C	100	150	100	124	100	132	100	122	100	139	100	12	100	113
N	274	203	82	21	60	21	17	16	6	3	73	0	28	13

Activities expressed in relative values taking that of normal liver as 100. Shaded areas represent values for fasted liver and are corrected for changes in protein content so that comparisons are effectively per cell and per nucleus.

TABLE VI

Intracellular Distribution of Enzymes in Fetal and Adult Intestinal Calf Mucosa

	Adenosine deaminase		Alkaline phosphatase		Esterase		Nucleoside phosphorylase		β-Glucuronidase		Liver glucuronidase	
C	3	127	116	344	0.238	0.336	7	19	0.344	0.410	3.64	1.88
N	1.9	24	5	12	0.067	0.029	6.3	4.5	0.092	0.102	0.891	0.324
100 N/C	63	19	4.3	3.5	28	8.5	90	24	27	25	25	17

(Shaded areas represent values for adult tissue.)

One result of this comparison was the observation that lipase and amylase, which are so highly active in the adult pancreas, are barely detectable in the fetus. The activity of these enzymes would thus appear to be adaptive. Cells of the intestinal mucosa, on the other hand, lend themselves to a different comparative study. Their biological history is singular. In the adult, the cells live vigorously but briefly; desquamation is a continuous process. If one scrapes the wall of the intestine for these cells, the spectrum of types ranges from those barely started on their brief career to those just ended. Desquamation, to be sure, also occurs in the fetus, but presumably at a less accelerated pace. In comparing adult and fetal mucosa, the contrast is thus between cells about to die and those with something of a life expectancy. The observed difference in enzymatic pattern of these two tissues (summarized in Table VI) certainly parallels the known difference in physiological state. The cytoplasm of the adult tissue, for example, has forty times as much adenosine deaminase activity as the fetal tissue. Increased activities in the adult cytoplasm were observed for

all enzymes studied. On the other hand, the relative nuclear activities (N/C) are higher in the fetal mucosa than in the adult. Only β-glucuronidase activity constitutes an exception to the general decline in N/C ratio from fetus to adult. (The values for nuclear phosphatase are not considered because they are within the range allowed for error of contamination.) An additional point of interest is the fact that fetal mucosa cells are much the same as liver cells or other adult cells with respect to their intracellular distribution of nucleoside phosphorylase and adenosine deaminase. It will be recalled that adult mucosa tissue constituted the only exception to the otherwise high nuclear concentration of these

TABLE VII

Nucleoside Related Enzymes of Adult and Fetal Calf Tissues

		Liver		Pancreas		Kidney	
Nucleoside phosphorylase	C	26*	52	18*	28	15	14
	N	23	52	30	21	15	19
	100 N/C	90	100	165	75	100	136
Adenosine deaminase	C	210*	52	0.94*	0.81	7*	Trace
	N	123	108	1.90	1.32	9	
	100 N/C	59	208	203	163	130	

* Values for whole tissue.

enzymes. The temptation is strong to view the low nuclear phosphorylase and deaminase activity as a concomitant of moribundity. Indeed one might say, that in cells destined to short life, as in cells subjected to starvation, the relative activity of the nucleus falls. The exception of β-glucuronidase behavior is thus of special interest. From Table V it can be seen that in starvation there was little change in the relative nuclear activity of this enzyme; the parallel with starvation is to this extent complete. Why β-glucuronidase activity in the nucleus should show such comparative stability remains unclear. Nor can an explanation be offered for its rise in mucosa and its decrease in the liver upon maturation.

A very different aspect of the comparative properties of adult and fetal tissues is provided by the adenosine deaminase and nucleoside phosphorylase

activities of organs like the liver, kidney, and pancreas (Table VII). In these instances, one compares a growing tissue with one fully grown, but hardly short lived as in the case of the adult mucosa. The enzymatic contrasts are correspondingly not as marked, nor is the pattern of behavior regular. The one consistency observed in both fetus and adult is that already noted for all adult tissues; namely, that the activity of these enzymes shows a variable but high nuclear concentration.

DISCUSSION

In the various speculations designed to explain the role of the nucleus in the differentiation of an organism, two aspects of nuclear behavior have been generally stressed. On the one hand, in light of cytogenetic evidence for chromosomal continuity, the idea has been formulated that nuclei, irrespective of tissue of origin, are of much the same composition (23). On the other hand, based upon the observations of early cytologists that nuclei vary morphologically in the course of glandular activity (22), the idea of a variable nucleus has been commonly accepted. These two ideas have usually been regarded as compatible since the variability observed was taken to mean that nuclei might be more or less active, but that the over-all pattern of nuclear composition did not change from tissue to tissue. This view of nuclear composition has had, however, only one point of basis in chemical fact—the constancy in the amount of DNA per somatic nucleus. The microspectrophotometric studies of Caspersson and coworkers (1) have restated the phenomenon of variability in terms of a generalized nucleoprotein metabolism, but, of necessity, have not dealt with the question of nuclear constancy. Only with the elaboration of chemical studies on isolated nuclei has a more comprehensive consideration of this question become possible. Dounce (2) in consequence of such studies, has pointed to the variability of nuclei. The results presented here emphasize not only nuclear variability, but equally, nuclear differentiation. The conclusion is inescapable because of the fact that some cellular components—hemoglobin, arginase, catalase,—which are restricted to certain tissues of an organism, are to be found concentrated in the nuclei of only those tissues. The extent to which a special component may be concentrated in the nucleus is illustrated by hemoglobin, which constitutes more than half of the non-nucleohistone protein of the avian erythrocyte nucleus. Furthermore, with respect to enzymes of general distribution, the nuclei show as great a variability as the tissues themselves. The conception that nuclei of a species have a similar composition, irrespective of tissue of origin, has therefore no basis in chemical fact.

The main implication of the commonly held conception of nuclear constancy is that differentiation is limited to the cytoplasm. To explain the phenomenon of a differentiating cell, two strong lines of evidence have had to be taken into account—that of embryology pointing to the cytoplasm as seat of the original

stimuli to the process, and that of genetics pointing to the nucleus as influencing and limiting the diverse events of the cell. A simple setting which could thus be provided for the differentiation of tissues was one in which the primary and approximately constant gene products were selectively activated by a variable cytoplasmic substrate. Essentially, this was the view of Goldschmidt (24) which, though modified in various ways, has commonly given rise to the diagrammatic representation of nucleocytoplasmic interaction in terms of a one-way arrow leading from nucleus to cytoplasm. To reconcile such a representation of chromosome activity in a constant intranuclear environment, with the evidence presented here for differentiation of nuclei, one would have to suppose that the observed differences in nuclear composition were merely the result of a *passive* diffusion of components from cytoplasm to nucleus unrelated to the requirements of either nuclear metabolism or the cell as a whole. That this is unlikely from a general biological standpoint should be apparent, but it is worth emphasizing that in the entire pattern of distribution observed, the physical factors likeliest to be associated with such a diffusion process—solubility and molecular size—bear no relation to the actual presence or absence in the nucleus of the various components (see pp. 563–565). Furthermore, it is clear that passive diffusion tends to an equalization of concentrations; no such tendency has been observed. In fact, where enzymes are more concentrated in the nucleus than in the cytoplasm, or where, in the case of starvation, changes in concentration run oppositely in cytoplasm and nucleus, the occurrence of a mechanism of passive diffusion cannot be postulated. If so, the presence of special cytoplasmic components within the nucleus must be considered as an active and hence, necessary part of nuclear metabolism. Nucleocytoplasmic interaction would thus appear to involve a reciprocity of exchanges in which, apart from the movement of nutrient metabolites, cytoplasmic components modify the intranuclear environment and nuclear products mediate events of the cytoplasm. Why many of the "special" components are absent from the nuclei of adult tissues is a problem, the solution of which requires some knowledge of the intracellular distribution of these components in the developing embryo.

Apart from the relevance of nuclear enzymatic activity to the process of differentiation, there exists the related question of the significance of such activity in the functioning of the mature cell. In recent years a great deal of attention has been focussed on the intracellular distribution of enzymes, not only in relation to nuclei, but also in relation to various particulate cytoplasmic structures which can be isolated by differential centrifugation. The aim of these studies has been to determine the enzymatic functions of these different intracellular structures and their contribution to the over-all metabolism of the cell. The localization of an enzyme in an intracellular structure is commonly regarded as functionally significant only when two criteria are satisfied: First,

the concentration of the enzyme in the isolated particulate fraction must exceed its concentration in the original tissue homogenate, and, secondly, the total activity of the particulate fraction must comprise a significant and preferably a major proportion of the total activity of the tissue. (This latter criterion also requires that the additive activities of the different fractions correspond to the initial activity of the tissue suspension.) In this way it was demonstrated that both cytochrome oxidase and succinic dehydrogenase are localized in the mitochondria (25). This "balance sheet" computation of enzyme distribution has advantages in detecting contamination and in discovering localized inhibition of enzymatic activity. It has, however, a more deep seated implication which makes its general application to enzymatic studies of cell fractions questionable. In calculating the "contributions" of the individual cell fractions to the activity of the whole cell, or homogenate, the assumption is made that each enzyme has a uniform significance regardless of the structure in which it occurs. Thus in isolated nuclei, even an enzyme *concentration* in excess of that measured for the cell would be regarded as of doubtful significance, or of secondary importance, if the total activity of the nuclear fraction was only a small proportion of the activity of the tissue as a whole. Genetic evidence, on the other hand, is so overwhelming for the dominating influence of the nucleus on cellular function, that it is inconceivable that the significance of enzymes which are highly active in the nucleus should be merely in proportion to their contribution to the total activity of these enzymes in the cell as a whole. Unless one regards nuclear metabolism as a cloistered mechanism mediating its influence on the cytoplasm entirely by substances of uncommon, and yet unknown nature—and there is no reason to believe that this is so—the activity of enzymes in nuclei must have a special significance to both nuclear and cytoplasmic function.

The fact that nucleoside-specific enzymes are usually concentrated in the nucleus provides ample reason for believing that they have a particular importance to nuclear activity. One might speculate, for example, in view of the role which nucleoside phosphorylase plays in the synthesis of DPN, that the nucleus exerts a specific control on the synthesis of this and other coenzymes. One cannot yet tell, however, whether the nucleus is a site of synthesis for the enzyme itself, or whether the nuclear enzyme mediates the synthesis of coenzymes, or metabolizes some unique nuclear substrate. It is with a resolution of such problems that part of future investigations will be concerned.

SUMMARY

The composition of isolated nuclei and cell preparations from tissues of calf, beef, horse, and fowl was studied with respect to the following components:

1. Liver and kidney arginase, catalase, and uricase; pancreatic lipase and amylase; cardiac muscle myoglobin; erythrocyte hemoglobin; intestinal alkaline phospharase. These are referred to as "special" components in view of their

characteristically restricted distribution reflecting the differentiated nature of the tissues in question.

2. Esterase, β-glucuronidase, alkaline and nucleotide phosphatases, adenosine deaminase, guanase, and nucleoside phosphorylase. These are enzymes of general distribution.

The differences in nuclear composition noted with respect to the "special" components, together with the broad variability in nuclear activity found for enzymes of general distribution, led to the conclusion that nuclei are differentiated structures.

The following distribution was observed:

1. "Special" components: Hemoglobin was found to be present in fowl and goose erythrocyte nuclei, but myoglobin was entirely absent from heart muscle nuclei; of the special enzymes listed, only catalase and arginase appeared to be concentrated in some of the nuclei. There was no significant nuclear concentration of lipase, amylase, uricase, or alkaline phosphatase. No simple relationship was found between the concentration of a special enzyme in a tissue and its activity in the corresponding nuclei. For example, arginase activity, which is high in mammalian liver and in fowl kidney, was found in liver, not kidney, nuclei. Similarly, catalase activity was demonstrated only in mammalian liver nuclei, although, in mammals, both liver and kidney are rich sources of this enzyme.

2. Enzymes of general distribution fell into three classes:

(*a*) Those present in low concentrations, if at all, in the nuclei—alkaline phosphatase, the nucleotide phosphatases, and β-glucuronidase.

(*b*) Those present in nuclei in varying concentrations—esterase.

(*c*) Those present in high proportions in most nuclei—adenosine deaminase, nucleoside phosphorylase, and guanase. The exceptionally low nuclear activity of intestinal mucosa with respect to these enzymes was discussed in relation to physiological considerations.

The response of nuclei to changes in physiological state was demonstrated by experiments on starvation. The outstanding aspect of this response was a change in nuclear enzymatic activity opposing that observed in the cytoplasm.

A comparison of fetal and adult mucosa cells led to the following tentative interpretation of the observed intracellular enzyme distribution: In cells tending to moribundity, as in those subjected to starvation, relative nuclear enzymatic activity falls.

The occurrence of special enzymes in nuclei was considered in terms of differentiation, and the high nuclear concentration of the nucleoside-specific enzymes was interpreted in terms of general nuclear metabolic activity.

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