SOLUBLE ENZYMES OF NUCLEI ISOLATED IN SUCROSE AND NON-AQUEOUS MEDIA

A Comparative Study

By HERBERT STERN and A. E. MIRSKY

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

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This paper is concerned with the behavior of sucrose-isolated nuclei in respect of soluble proteins; it is the object of the present report to determine whether or not this aqueous procedure can be used in studies of soluble components of nuclei.

Isolation of nuclei in sucrose is relatively quick and convenient; it is, in fact, commonly regarded as the procedure of choice. Considering the generality of its use, it is remarkable that the reservations occasionally expressed concerning the soundness of the procedure (1) have held a mute second place to the lively assertions based upon this very procedure concerning the composition of nuclei in respect of water-soluble components, most particularly enzymes (1, 2). To the writers' knowledge no definitive attempt has been made to ascertain the limits of the technique in question. The need for knowing these limits is, however, patent, and to this end we have attacked the problem in a most direct way; nuclei isolated by the Behrens technique in which washing out of proteins is virtually impossible are compared with nuclei of the same tissue prepared in sucrose solution.

In thus considering the sucrose technique no attention is given to the low speed sediment commonly called the "nuclear fraction." Our experience has been consistently to find it so undefinable a mixture of whole cells, cell fragments, assorted debris, and nuclei that, properly regarded, it is no more than a tissue fraction containing nuclei. The properties of this fraction have no necessary connection with nuclei and, therefore, admit of no reasonable inferences respecting their enzymatic activities. Our interest lies only in those sucrose preparations which yield at least moderately clean suspensions of nuclei. In these, the problem can be restricted to the principal question: Do nuclei isolated in sucrose retain their complement of soluble enzymes? Hogeboom and Schneider (3) believe that at least in rat liver the nuclear membrane protects against outward diffusion of protein. Anderson (4) believes the evidence to point to a porous membrane and identifies such porosity with the *in situ* condition of the nucleus. He presses no argument, however, for limiting the use of sucrose media and assembles no data bearing on its unreliability. It will be

shown in this report that studies of calf thymus and liver establish the equivocal nature of data on soluble enzyme composition which are drawn from nuclei isolated in sucrose media.

Methods

Thymus or liver from a freshly killed calf was placed quickly on ice and brought immediately to the laboratory, the time interval involved not exceeding an hour. Thymus tissue was divided in three parts and treated in these ways: (1) A portion was frozen quickly over dry ice and then lyophilized; this was used to prepare nuclei by the modified technique of Behrens (5). (2) A 25 per cent suspension of whole tissue in 0.25 M sucrose–0.0018 M $CaCl_2$ was prepared in a blendor. A small aliquot was set aside for immediate analyses and the remainder was frozen with dry ice–acetone mixture and lyophilized. (3) 75 gm. of tissue was homogenized with 75 ml. of 0.5 M sucrose–0.0018 M $CaCl_2$ plus 600 ml. of 0.25 M sucrose–0.0018 $CaCl_2$ in a Waring blendor at 35 volts for 4 minutes, the suspension strained through a double layer of surgical gauze and again through a layer of double napped flannelette (6). The filtered suspension was then spun in a horizontal centrifuge for 10 minutes at 600 *g*. The resulting sediment was resuspended in 200 ml. of sucrose-$CaCl_2$ solution with the aid of a glass rod. A variable amount of the suspension clumped and this was allowed to settle on standing. The supernatant was then decanted, the suspension again centrifuged, and the sediment resuspended in a convenient volume of sucrose-$CaCl_2$ solution. An aliquot of this suspension was set aside for immediate analyses and the remainder frozen with dry ice–acetone mixture and lyophilized. The two lyophilized preparations were treated for 24 hours with a 2:1:1 mixture of petroleum ether: cyclohexane:carbon tetrachloride in order to parallel in treatment nuclei obtained by the Behrens procedure.

Calf liver nuclei were prepared by first mincing 200 gm. of fresh tissue in a meat grinder and washing this repeatedly with cold isotonic sucrose to remove most of the blood. A portion of the tissue was made up as a 25 per cent suspension and treated exactly as that of thymus. The remainer was suspended in 300 ml. of 0.25 M sucrose–0.0018 M $CaCl_2$ and blended as thymus but for three 5 minute intervals. The resulting suspension was filtered through gauze and blended again for 7 minutes. Despite this treatment an appreciable number of whole cells remained. Nuclei were first sedimented by centrifuging the suspension at 600 *g*. The sediment was resuspended and the operation repeated twice. Following this the nuclear fraction was layered over 0.34 M sucrose as described by Hogeboom *et al.* (7). The resulting sediment was then resuspended in a convenient volume of 0.25 M sucrose–$CaCl_2$ and treated as were the nuclei of calf thymus.

For enzyme assays, extracts of fresh sucrose suspensions were prepared by diluting these 5 to 20 times with water and then made 0.1 M with respect to K_2HPO_4; lyophilized preparations were homogenized directly in K_2HPO_4. All tissues were extracted for 4 to 6 hours in the cold to yield upon centrifugation a clear or nearly clear supernatant.

Activities are referred to the N content of the tissue extracted, not that of the extract. All measurements were spectrophotometric and assays were conveniently per-

formed in cuvettes at room temperature (approximately 25°C.). Components of the reaction mixtures and units of activity were as follows:—

1. Glucose-6-phosphate dehydrogenase: 0.05 ml. phosphate buffer (1.5 M, pH 7.5); 0.1 ml. glucose-6-phosphate (0.05 M); 0.1 ml. triphosphopyridinenucleotide (0.5 mg./ml. of Sigma TPN "80"); 0.65 ml. of tissue plus water.

Activity: ΔE_{340}/mg. N/minute (Readings taken for 5 minutes at 1 minute intervals).

2. Adenosine deaminase: 0.1 ml. phosphate buffer (1.5 M, pH 7.5); 0.1 ml. adenosine (200 γ/ml.); 2.8 ml. tissue plus water. Readings taken every 2 minutes for 10 minutes at 265 and 248 mμ (8).

Activity: γ adenosine deaminated/mg. N/10 minutes.

3. Nucleoside phosphorylase: 0.05 ml. phosphate buffer (1.5 M, pH 7.5); 0.02 ml. xanthine oxidase; 0.06 ml. inosine (200 γ/ml.); 0.87 ml. tissue plus water. Readings taken every 2 minutes for 10 minutes at 290 and 272 mμ (9).

Activity: γ hypoxanthine liberated/mg. N/10 minutes.

Xanthine oxidase was prepared according to the method of Ball (10) and the product stored at 0°C. under saturated ammonium sulfate. For use enough of the paste was dissolved in about 1 cc. of water to yield a pale yellow solution.

4. Alkaline phosphatase: Suspensions of thymus were prepared by homogenizing treated tissues in 0.25 M sucrose. With care such suspensions could be used in direct spectrophotometric assay, phenolphthalein phosphate being the substrate (11). Components of the reaction mixture were as follows: 2.0 ml. buffer substrate mixture (304 mg. sodium phenolphthalein phosphate, 2.65 gm. Na_2CO_3, 2.1 gm. $NaHCO_3$ in 500 ml. water); 1 ml. tissue plus water. Readings were taken every 2 minutes at 553 mμ for 10 to 20 minutes.

Activity: ΔE_{553}/mg. N/1 minute.

DNA analysis: Weighed portions of lyophilized sucrose preparations of nuclei were washed with 95 per cent alcohol at 60°C. to remove the sucrose. Overnight extraction was found necessary to ensure complete removal. The tissue was then washed with ether and dried at 100°C. Tissue thus treated was again weighed and the DNA content determined by the diphenylamine reaction.

RESULTS

A. Calf Thymus

Protein Content of Sucrose-Prepared Nuclei.—The DNA/N ratios, or alternatively, the percentage DNA contents of nuclei obtained by aqueous and non-aqueous procedures constitute a most general basis of comparison for determining the protein lost or gained in the course of aqueous isolation. For any meaningful calculation of the protein content, however, the preparation of nuclei must be microscopically *very* clean. In absence of this condition, an indeterminate amount of protein contaminant will serve variably to balance an indeterminate amount of protein which might have been washed out of the nuclei in the course of their preparation. Cytochrome oxidase measurements alone are not enough. Both rat liver and calf thymus nuclei showed virtually

no cytochrome oxidase activity (measured spectrophotometrically) yet, in our hands, rat liver nuclei, even those yielding the best DNA/N ratios claimed by Hogeboom *et al.* (7), appeared to be the much inferior preparation microscopically. The thymus nuclei were not only more satisfactory in appearance but except for a very small number of whole cells showed little of any other type of contaminant. We considered that the thymus preparation provided a reliable index of the amount of protein retained by the nuclei in the course of their preparation in an aqueous medium.

The DNA-P contents of nuclei prepared in sucrose and in non-aqueous media were 2.33 and 2.21 per cent, respectively. These values are much the same and it is reasonable, therefore, to conclude that in thymus nuclei no net loss of protein occurs when these are prepared in sucrose media.

TABLE I

Effect of DNAase on Thymus Nuclei Isolated in Sucrose Solution

Time	E_{260}		Per cent of total DNA degraded
	Control	Plus DNAase	
hrs.			
1	0.200	1.43	15
2	0.225	2.05	22
4	0.255	2.81	30
5½	0.260	3.25	34
18	0.375	4.50	47

Permeability of Isolated Nuclei.—Anderson (12) has shown that rat liver nuclei isolated in sucrose are permeable to desoxyribonuclease. Since protein appears to be retained in thymus nuclei the question naturally arises as to whether such retention is a function of the state of the nuclear membrane. To this end the experiment following was performed.

4 cc. of a sucrose suspension of nuclei containing 2.74 mg. acid-insoluble N/cc. was diluted with 1 cc. of "Tris" buffer (0.125 M, pH 7.4) containing also sucrose (0.25 M) and $CaCl_2$ (0.0018 M). Two such suspensions were made, one being retained as a control, while to the other was added 0.16 cc. of 1.0 M $MgSO_4$ plus a small quantity of DNAase (a crystalline sample kindly furnished by Dr. Kunitz). Periodically 0.8 cc. samples were withdrawn and centrifuged to yield a clear supernatant; an aliquot of this was diluted, eightfold in case of the control and 40-fold in case of the DNAase-treated sample, for extinction measurements at 260 mμ. The results of such readings calculated for eightfold dilutions are given in Table I.

The evidence for the penetration of the DNA is shown not only quantitatively in the progressive release of soluble nucleotides, but the qualitative

difference between control and experimental suspensions is impressive. After standing at 0°C. for 18 hours the control nuclei were stuck together in a clump and could not be separated easily; the DNAase-treated nuclei were settled at the bottom of the tube but could be easily dispersed. Furthermore, it may be safely assumed that undenatured nucleoprotein will limit the extent to which the DNA moiety is attacked by the enzyme. Thus, it is almost certain that the apparent protection of half of the total DNA against the activity of the enzyme is due to intranuclear conditions and not to the state of the nuclear membrane.

Enzyme Properties of Isolated Nuclei.—In a comparative study aimed at fixing retention or loss of protein, data on enzyme activity confer a distinct advantage; they treat of individual proteins and are sensitive beyond the reach of nitrogen analyses. Such data, nevertheless, must be treated with caution since the concentration of an enzyme in crude extracts cannot always be directly equated with the activity measured. Moreover, the extreme differences

TABLE II

Effect of Lyophilization and Treatment with Organic Solvents on Enzyme Activities of Sucrose-Suspended Tissues of Calf Thymus

Enzyme	Whole tissue		Nuclei	
	Fresh	Treated	Fresh	Treated
Glucose-6-phosphate dehydrogenase........	0.082	0.170	0.059	0.240
Adenosine deaminase....................	1350	1950	513	1745
Nucleoside phosphorylase.................	36	66	16	40

Units of activity for each of the enzymes listed indicated under "Methods."

in treatment of nuclei derived by the Behrens and sucrose procedures begged a comparison of the effects of such treatments on enzymatic activity.

In Table II are summarized the results of a series of comparisons between tissues differently treated. From these we infer the following:

1. The lyophilization of thymus tissue and its subsequent treatment with organic solvents do not decrease enzyme activity from the values obtained in extracts of freshly blended preparations. It is unlikely, therefore, that the enzymes in question are even partially inactivated by treatment.

2. In all cases, tissues which have first been homogenized in sucrose and then lyophilized show marked increases in activity. This effect is in part due to the presence of sucrose since tissues frozen directly are closer in properties to the fresh preparations.

3. Sucrose nuclei, if lyophilized, show the biggest increase in activity, this being as much as fourfold in the case of glucose-6-phosphate dehydrogenase. We consider, therefore, that probably the better comparison of the aqueous and non-aqueous procedures of preparing nuclei—and certainly the one favor-

ing the aqueous method—is made with sucrose-homogenized tissue lyophilized and then treated with organic solvents. There is, of course, the added factor of convenience in being able to store the preparations obtained.

4. Although the increased activity of tissues suggests some activation phenomenon, there is reason to believe that the differences may be due to degree of extraction. Lyophilized sucrose tissue yields the finer suspension upon homogenization in phosphate solution, and in the case of thymus with its very high content of DNA tending to formation of a gummy mass, this might be of importance. Furthermore, similar treatment of sucrose-isolated rat liver showed no corresponding increases in activity.

If lyophilized and treated nuclei prepared by the aqueous and non-aqueous procedures respectively are compared in terms of enzyme activity (Table III), the results are clear enough. There is no significant washing out of enzymes. Among the soluble enzymes, it may be noted that glucose-6-phosphate dehydrogenase is possibly in part adsorbed to the nuclei from the soluble fraction (that this enzyme is in the soluble fraction may be easily shown by testing the supernatant after centrifuging to remove the microsomal fraction), but this possibility is better considered in light of results on liver nuclei. Alkaline phosphatase is consistent in its distribution in both types of preparations. Since we have obtained much the same results in a number of aqueous and non-aqueous preparations, it appears reasonable to conclude that in thymus tissue the use of sucrose media for nuclear isolation does not involve a serious washing out of protein.

TABLE III

Enzyme Activities of Thymus Nuclei Isolated in Sucrose and in Non-Aqueous Media

Enzyme	Sucrose		Non-aqueous		$N/W \times 100$	
	Whole tissue	Nuclei	Whole tissue	Nuclei	Aqueous	Non-aqueous
Glucose-6-phosphate dehydrogenase	0.170	0.240	0.080	0.069	140	87
Adenosine deaminase	1950	1745	1350	1350	90	100
Nucleoside phosphorylase	66	40	60	28	60	47
Alkaline phosphatase	0.068	0.012	0.048	0.003	18	6

Units of activity for each of the enzymes listed indicated under "Methods."

B. Rat and Calf Liver

By themselves, the results on thymus would encourage the use of the sucrose procedure for all types of study. Data on rat liver nuclei isolated in sucrose (13), however, make these results suspect. Fresh preparations of such nuclei show virtually no nucleoside phosphorylase or adenosine deaminase activity;

with respect to glucose-6-phosphate dehydrogenase our measurements showed the nuceli to be one-fourth as active as the whole tissue. Since not only thymus but almost all tissues we have examined had relatively high concentrations of these soluble enzymes in their nuclei (14), the question naturally arose as to whether rat liver nuclei constituted some genuine exception. The situation is possible, but from a physiological standpoint improbable.

The possibility that rat liver nuclei isolated in sucrose solution might become active enzymatically if treated in the same way as the nuclei of thymus was tested. Neither of the enzymes measured, however—nucleoside phosphorylase and glucose dehydrogenase—showed increases significant enough to alter the picture. The next step was, therefore, to prepare either rat liver nuclei by the non-aqueous method, or calf liver nuclei by the sucrose procedure. We chose the second alternative because clean preparations of rat liver nuclei by the non-aqueous method are difficult to obtain.

TABLE IV

Enzyme Activities of Calf Liver Nuclei Isolated in Aqueous and Non-Aqueous Media

Enzyme	Sucrose		Non-aqueous		$N/W \times 100$	
	Whole tissue	Nuclei	Whole tissue	Nuclei	Aqueous	Non-aqueous
Glucose-6-phosphate dehydrogenase...	0.119	0.063	0.105	0.098	53	94
Nucleoside phosphorylase............	313	14	365	365	4	100
Adenosine deaminase..............	1140	231	1210	2520	20	208

Units of activity for each of the enzymes listed indicated under "Methods."

Sucrose nuclei prepared from calf liver are poor and mainly in this respect; they are appreciably contaminated with whole cells. It has already been indicated in the description of method that the calf liver was blended for a prolonged period of time but even so the presence of whole cells plus much debris could not be eliminated. The poorness of the preparation nevertheless served to emphasize a point: If a nuclear fraction contaminated in part by whole cells failed to show soluble enzyme activity, then most certainly a pure preparation of such nuclei would not.

The DNA-P content of the treated nuclear fraction used in these studies was 1 per cent, that of nuclei prepared by the non-aqueous technique was 1.5 (5). The nuclear content of the fraction was, therefore, no more than approximately 65 per cent. The contrast, however, between the properties of the two preparations with respect to soluble enzyme activity leaves no doubt as to the nature of the effect of using sucrose in preparing nuclei (Table IV). Adenosine deaminase and nucleoside phosphorylase are washed out; glucose phosphate dehydrogenase alone is retained in an appreciable amount. This latter fact is,

however, suggestive. In thymus nuclei when soluble enzymes are retained, dehydrogenase is relatively much more concentrated in the nuclei prepared in aqueous media than in those prepared by the non-aqueous method; in calf and rat liver, when the soluble enzymes are washed out, only dehydrogenase is retained. In view of this, it appears probable that some dehydrogenase tends to be adsorbed by the nuclei when the tissues are homogenized in a sucrose medium.

DISCUSSION

The principal question which was originally set has been clearly answered: Nuclei isolated in sucrose media cannot be reliably used for determining the intracellular distribution of soluble enzymes. Not only is the evidence direct for the washing out of enzymes, but if proteins can move out they can also move in and, therefore, adsorption at surfaces other than the nuclear membrane must be taken into account. In connection with adsorption, the evidence has already been discussed for glucose phosphate dehydrogenase being partly thus retained in isolated nuclei. There is little doubt that other enzymes could be similarly adsorbed to a greater or lesser extent, depending on the one hand upon the composition of the nucleus and on the other, upon the chemical properties of the enzyme. These facts compel a clear position: Analyses of sucrose-isolated nuclei for soluble protein composition admit only of possible conclusions, the results of such analyses falling anywhere between the extremes of perfect reliability and pure error. Such characteristics make the use of a method inadmissible by any scientific standard. As for the "nuclear fraction" alluded to in the introduction, it can be considered no more than a compounding of errors.

Much has occasionally been made of the appearance of nuclei in sucrose solutions as evidence for the favorableness of the medium. To be sure, the extreme differences between the highly refractile properties of a coagulated cell and the optical homogeneity of a living cell nucleus lend themselves to unambiguous interpretation. It does not follow, however, that the degree of similarity in optical property between isolated and *in situ* nuclei can be taken as measure of the extent of their correspondence in physiological state. Ris and Mirsky (15) have pointedly shown that the appearance of an interphase nucleus depends largely on the physical state of the DNA moiety of the chromosomes, and that in non-electrolytes such as glycerine or sucrose the chromosomes remain extended and the nucleus appears homogeneous. In the presence of a small amount of electrolyte the chromosomes can be made to condense; since this condensation is reversible the same nucleus can be made alternately to resemble or to differ from that in the intact cell. Such behavior hardly encourages the use of optical property as anything more than a rough index to the structural intactness of an isolated nucleus.

The criticism attached to the use of sucrose in respect of soluble protein may seem severe in the light of its common and successful use in cell physiology.

The point made, nevertheless, is a most specific one covering only the retention or adsorption of soluble components in isolated nuclei; the relation of the sucrose medium to the maintenance of certain structural characteristics native to the *in situ* nucleus is not brought into question. That sucrose can stabilize protoplasts has been shown in the case of dividing plant cells (16). It was, moreover, demonstrated that the actual penetration of sucrose into the cell and not its effect on the permeability of the cell membrane was responsible for the favorable effects of the sucrose medium. Whether the preservation—or destruction—of vital structural properties is possible with isolated nuclei is beyond the scope of this investigation and at present still constitutes an open field for study. In connection with the internal structure of sucrose-isolated nuclei it is possible only to conjecture on the difference between those of thymus and liver. Both are permeable to DNAase, a protein of 60,000 molecular weight (17), and there are, therefore, good grounds to assume that the retention of enzymes by thymus nuclei is not related to permeability properties of the nuclear membrane. The difference, however, between their respective contents of DNA is appreciable; calf thymus nuclei have a DNA content of 26 per cent whereas those of calf liver have only 15 (5). It seems plausible, therefore, to suppose that thymus nuclei would tend to form a more coherent gel in sucrose medium and thus retain most, if not all, of the soluble protein. Whatever the nature of the difference it is clear that the only way to ascertain the reliability of analyses for soluble components in sucrose-isolated nuclei is by checking with a non-aqueous preparation.

The present results also invite a point of interpretation which we here wish to emphasize. It is possible to view the free permeability of the isolated nucleus to protein as actually representing the condition obtaining in the *in situ* nucleus. Given this, it is possible further to construct a picture of nucleocytoplasmic interaction in which the soluble proteins of the cell constitute a "continuous phase" moving freely through a non-selective nuclear envelope—a membrane real enough morphologically but of limited function physiologically. Although admitting the caution with which inferences from sucrose-isolated nuclei must be treated, this is the possibility elaborated at length by Anderson (4). The embarrassments of data showing unequal distribution of many soluble components between nucleus and cytoplasm or even their total absence from the nucleus, are relieved by supposing that adsorption to particulate constituents would remove a variable amount of a component from the soluble phase of the equilibrium system. The crucial point, as viewed by Anderson, is how to explain the evidence, drawn from intact cells, that there is an exchange of macromolecules between nucleus and cytoplasm if the nuclear membrane is presumed to be "semipermeable." In face of this, Anderson sides with the interpretation that the permeability of the isolated nucleus (in sucrose) is the permeability of the *in situ* one.

It is questionable, however, whether "semipermeability" of a membrane and the passage of macromolecules across it are incompatible. If a parallel is drawn from the many studies of "active transport" across cell membranes, it would appear quite probable for the nuclear membrane to act structurally as a barrier to free diffusion between nucleus and cytoplasm while functioning by energy-requiring mechanisms in the transportation of substances to and from the nucleus. From this point of view, the nucleus isolated in sucrose is an injured nucleus, at least in so far as the membrane is concerned. The available facts do not, of course, compel a conclusion either way concerning the natural state of the nuclear membrane; they do, however, compel a conclusion concerning the use of sucrose media for determining the composition of nuclei in respect of soluble components. It is noteworthy that Anderson (4) treats equally the demonstration of glycolytic enzymes in nuclei prepared in sucrose and those prepared in non-aqueous media; in light of his arguments this hardly seems justified whatever the natural state of the nuclear membrane.

SUMMARY

Nuclei of calf thymus and liver and of rat liver were isolated in sucrose media and a number of their properties studied in relation to those of corresponding nuclei isolated in non-aqueous media with a view to determining their capacity to retain soluble components.

The best preparations of sucrose nuclei were obtained from calf thymus. Cytochrome oxidase measurements and DNA/N ratios were far less sensitive than microscopic examination as indicators of purity when rat liver and calf thymus nuclei were compared. No satisfactory preparation of calf liver nuclei was obtained, contamination with whole cells having been appreciable; such preparations, nevertheless, could be used to advantage in the tests undertaken.

DNA content of thymus nuclei isolated in sucrose was much the same as that of non-aqueous ones, pointing to a retention of soluble protein under aqueous conditions of isolation. That this net retention of protein was not due to the impermeability of the nuclear membrane was shown by the hydrolysis of the DNA upon addition of some crystalline DNAase to a sucrose suspension of nuclei.

A comparative study of liver and thymus nuclei isolated in aqueous and non-aqueous media with respect to the soluble enzymes glucose-6-phosphate dehydrogenase, adenosine deaminase, and nucleoside phosphorylase yielded the following results:

1. Lyophilization of sucrose-isolated nuclei and their extraction with the organic solvents used in the non-aqueous procedure did not inactivate any of the enzymes tested. In the case of thymus the reverse was true, there being a marked increase in activity of all the enzymes studied.

2. In thymus, nucleoside phosphorylase and adenosine deaminase were

active to approximately the same extent in nuclei isolated by either procedure. Glucose phosphate dehydrogenase alone was more active in sucrose-isolated nuclei, pointing to the possibility of an adsorption of this enzyme.

3. In rat liver nuclei isolated in sucrose, lyophilization and treatment with organic solvents revealed only the presence of some dehydrogenase.

4. The washing out of soluble enzymes was most markedly demonstrated in the case of calf liver. Only traces of the nucleoside enzymes were found in the sucrose-isolated nuclei, and in the case of the dehydrogenase only a half of that present in the non-aqueous nucleus remained.

The main conclusions drawn were as follows:—

1. In sucrose media the nuclear membrane is ineffectual in preventing the inward or outward diffusion of protein.

2. The extent to which soluble proteins are retained by a nucleus isolated in sucrose appears to depend upon internal structural factors, such as the concentration of DNA in the nucleus.

3. With respect to determining the composition of nuclei in terms of soluble components, the sucrose isolation procedure is considered to be of indifferent merit and hence invalid for such a type of analysis.

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