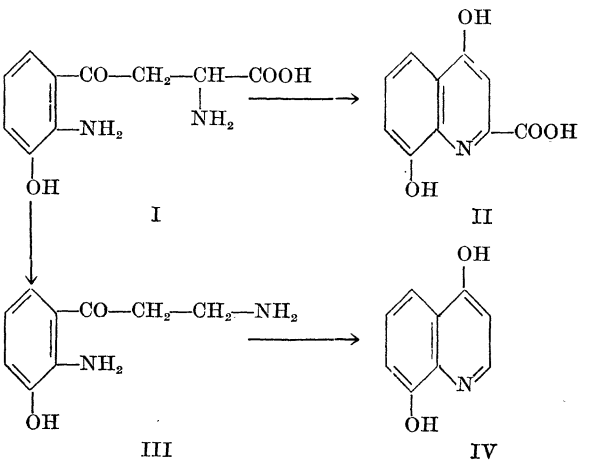
Table 1.

Sample \ Solvent	1	2	3	4	5	6	7	8
Substance A	0.79	0.79	0.04	0.69	0.64	0.09	0.68	0.75
4,8-Dihydroxyquinoline	.79	.78	.04	.68	.64	.09	.60	.77
Substance B	.45	.72	.00	.19	.05	.00	.15	.55
Xanthurenic acid	.45	.74	.00	.18	.05	.00	.15	.54

Solvent systems: (1) butanol, acetic acid, water (4 : 1 : 5); (2) methanol, butanol, benzene, water (4 : 2 : 2 : 2); (3) butanol saturated with 1-percent NH_4OH; (4) butanol saturated with 0.2-percent acetic acid; (5) iso-amyl alcohol saturated with 1-percent acetic acid; (6) iso-amyl alcohol saturated with 0.5-percent NH_4OH; (7) ethyl acetate, acetic acid water (5 : 1 : 5); (8) 2-percent acetic acid, isopropanol (3 : 7).

on exposure to the air and gave a vivid pink color with diazo-reagent. This behavior was the same as that of xanthurenic acid. So the two spots at Rf 0.79 (substance A) and Rf 0.45 (substance B) were more precisely identified by paper chromatography using seven other solvent systems, as indicated in Table 1, thus demonstrating complete coincidence of their Rf values with those of 4,8-dihydroxyquinoline and xanthurenic acid, respectively.

Since the incubation of xanthurenic acid (II) with mouse liver homogenate never led to the formation of 4,8-dihydroxyquinoline, the production of the latter from 3-hydroxykynurenine (I) may be considered to go by way of 3-hydroxykynurenamine (III), the oxidation of which seems to lead to 4,8-dihydroxyquinoline (IV), as follows.



References and Notes

1. K. Makino and H. Takahashi, XXVIth annual meeting of the Japanese Biochemical Society, 27 Apr. 1954.

2. This work was aided by the Scientific Research Fund of the Department of Education. We wish to thank T. Sakan for the gift of xanthurenic acid.

3. M. Kotake and T. Sakan, *Proc. Imp. Acad.* (*Tokyo*) **18**, 191 (1942).

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Communications

On the Intranuclear Environment

The success that has attended metabolic studies of mitochondria has naturally heightened desires in many cell physiologists to accomplish as much in the field of nuclear metabolism. Yet, in spite of all the studies of nuclear enzymatic composition, the results have been unimpressive. There is available a short list of the enzymes that have been classified as "nuclear," but, with one exception (a pyrophosphorylase), these enzymes are found in the cytoplasm as well. Hopes that nuclei, like mitochondria, would show a complement of uniquely localized enzymes, and thereby reveal directly the metabolic pattern of chromosome function, have remained unfulfilled. Some students of the problem, because of these results, have favored the conclusion that the interphase nucleus is the seat of few enzymatic catalyses. Other attitudes of mind have resisted the negative interpretation; occasionally a single enzyme has been chosen, and attempts have been made to wring out a special physiological significance from its presence in the nucleus. Alkaline phosphatase and adenosinetriphosphatase have been so used, but no workable perspectives of nuclear metabolism have resulted from such treatments. The most coherent—and probably the most fruitful—speculations on a pattern of nuclear metabolism have come, not from studies of nuclear enzymatic composition, but from studies of enucleation as interpreted by Brachet (*1*). In his scheme the nucleus is believed to exercise a direct and immediate influence on ribonucleic acid synthesis and phosphate esterification. Yet, the scheme skips, and does not solve, the question of an internal metabolic organization of the nucleus. To be sure, the results of present cytochemical research cannot provide a mechanism for the biochemical activities of the nucleus postulated by Brachet; but, if one avoids the objectives—borrowed from mitochondrial studies—of finding *the* important nuclear enzymes or of deciding the arithmetic contributions of the nucleus to the enzyme activities of the whole cell, some light can be thrown on the nature of the metabolic environment in which chromosomes operate.

Data on the intracellular distribution of enzymes point to the conclusion that chromosomes function in an anaerobic environment. It was first definitely demonstrated by Hogeboom *et al.* (*2*) that cytochrome oxidase is entirely absent from nuclei, a fact that has been shown to hold for a number of tissues. Thus, it

is reasonably certain that the particulate system responsible for the bulk of terminal oxidation is absent from nuclei. Flavoproteins, which may constitute either an autonomous aerobic pathway or a link in the aerobic chain between the pyridine nucleotides and the cytochromes, are also absent (*3, 4*). The latter situation is of special interest since flavoproteins, unlike cytochrome oxidase, are present not only in mitochondria but also in other structural elements of the cytoplasm. From the standpoint of cell organization (*2*), these coherent cytoplasmic elements offer a parallel with the corehent nuclear elements—the interphase chromosomes—but if the presence of nucleoproteins in each constitutes a point of similarity, the presence of flavoproteins in the one constitutes a marked point of difference. This is further underlined by the fact that cytochrome C, which is partly found in the soluble phase of the cytoplasm, presumably in contact with the more solid framework, cannot be detected in nuclei (*4*). Thus, while a potential chain for hydrogen transfer exists in the cytoplasm from the reduced pyridine nucleotides of the soluble phase, via the flavoprotein cytochrome C reductases of the coherent phase, through mobile cytochrome C to the cytochrome oxidase of the mitochondria, the reduced coenzymes of the nucleus must rely on other means for hydrogen transfer. This difference must be of importance since the respective capacities of nucleoplasm and cytoplasm to effect a primary breakdown of carbohydrate via triosephosphate or phosphogluconate are similar in concentration (*5*). Relative nuclear size is of little or no account in this matter, and in cells with large nucleo-cytoplasmic ratios (thymus, wheat germ), where the total production of reduced coenzyme from such primary carbohydrate oxidation may equal that of the cytoplasm, the absence of identifiable pathways for aerobic coenzyme reoxidation in the nucleus is given particular emphasis.

Support for the idea that chromosomes are adapted to metabolize in an anaerobic environment comes also from other sources. Nuclear division is a case in point, since, in the course of it, the chromosomes become exposed in the environment of the cytoplasm, albeit in a state more condensed than during interphase. Rapkine (*6*) had early pioneered to show that an increase in the concentration of reduced thiol groups was a precondition to cell division, thus offering the pointer that special conditions of hydrogen transfer might prevail during cell multiplication. Later, in studies of the anthers of *Lilium* and *Trillium,* where the relatively slow rate of division of the germinal tissue provides a material of choice for following sequential physiological changes, it was possible to demonstrate a relationship between the disappearance of the nuclear membrane and the appearance of a temporary phase of anaerobiosis (*7*). With present knowledge of mitochondrial function in terminal oxidation, the situation is understandable in light of the early observations of Lewis (*8*) that mitochondria become quiescent during nuclear division. The reciprocal of the foregoing relationship—that phases of nuclear division can proceed in the absence of oxygen—is also demonstrable. In depriving a growing mammalian tissue of oxygen, no effect is noted on cells that have already entered into mitosis (*9*). In polychaete eggs, inhibition of oxidation has no effect on the intranuclear formation of the ribonucleic acid and polysaccharide necessary for spindle development; under such anaerobic conditions nuclear division proceeds, but, by contrast, cytoplasmic division does not (*10*). The studies of Brachet (*11*) on nucleated and enucleated halves of amebas may also be cited: Under aerobic conditions both types maintain their ATP level equally well, but in the absence of oxygen the nucleated halves alone succeed, again suggesting that nuclear metabolism in the absence of oxygen proceeds without difficulty.

Assuming that the data is correctly interpreted, the question that obviously follows, "Why must chromosomes metabolize anaerobically?," is not easy to answer. One might suppose that, during the evolution of cell organization, oxidative mechanisms were available to nuclei as they were to other subcellular structures, and that the exclusion of oxidative mechanisms from nuclei conferred some evolutionary advantage on the genetic complex of the cell. The nature of this hypothetical advantage may be found in the somewhat opposite requirements for survival of genotype and phenotype, respectively. Survival of the phenotype is an aggressive process in which its capacity maximally to convert food to utilizable energy forms like adenosinetriphosphate (ATP) is of premium value in the course of growth; in this process terminal oxidation is a decided advantage. Given, however, the dynamic metabolic qualities of the host cell, the persistence of the genotype—however stable it might already be in consequence of its molecular organization—would appear to be better assured in a nucleus where volatile terminal oxidations are lacking and where carbohydrate breakdown, occurring at a more readily reversible level, subjects the genome to much less metabolic stress. In this connection, the finding of Conger and Fairchild (*12*) that oxygen causes chromosome breakage is of interest. ATP stores may be a secondary factor since the mediation of genetic influences depends to a large extent on the utilization of carbon chains (notably, the pentoses) that are more efficiently acquired at the expense of a limited carbohydrate metabolism of available hexoses than by CO_2 fixation at the expense of ATP. This is not to suggest that a reciprocity of substrate exchanges fails to take place between the nucleus and cytoplasm; it is meant to suggest that a measure of autonomy must be attached to the metabolism of the nucleus and that within the framework of this autonomy the operation of an anaerobic system is best suited to the metabolic requirements of the chromosomes. Along this line cytochemical studies have yielded promising information.

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References

1. J. Brachet, *Arch. biol. (Liége)* **65**, 1 (1954).
2. G. H. Hogeboom, W. C. Schneider, and M. J. Striebick, *Cancer Research* **13**, 617 (1953).
3. A. L. Dounce, *J. Cellular Comp. Physiol.* **39**, Supp. 2, 43 (1952).
4. H. Stern and S. Timonen, *J. Gen. Physiol.* **38**, 41 (1954).
5. H. Stern and A. E. Mirsky, *ibid.* **37**, 177 (1953).
6. L. Rapkine, *Ann. physiol. physicochim. biol.* **7**, 382 (1931),
7. R. O. Erickson, *Nature* **159**, 275 (1947); H. Stern and P. L. Kirk, *J. Gen. Physiol.* **31**, 243 (1948).
8. W. H. and M. R. Lewis, *General Cytology* (Univ. Chicago Press, Chicago, 1924), p. 383
9. W. S. Bullough, *Biol. Revs. Cambridge Phil. Soc.* **27**, 133 (1952).
10. H. Stich, *Experientia* **10**, 184 (1954).
11. J. Brachet, *Nature* **173**, 725 (1954).
12. A. D. Conger and L. M. Fairchild, *Proc. Natl. Acad. Sci. U.S.* **38**, 289 (1952).

20 December 1954.

Proximal or Distal Mercurial Inhibition of Succinic Dehydrogenase in the Kidney Tubules of Rat

In a recent histochemical paper (*1*) we reported that the administration of mercurophylline to rats resulted in an inhibition of the succinic dehydrogenase of the kidney, which was most pronounced in the thick ascending portions of Henle's loops. Later, Wachstein and Meisel (*2*) and Rennels and Ruskin (*3*) localized the inhibition of succinic dehydrogenase by mercuhydrin, another mercurial diuretic, in the proximal convoluted tubules. Wachstein and Meisel also stated that the ascending portions of Henle's loops retained their full activity. The highly divergent results prompted us to reinvestigate this question using both Novurit (*4*) (mercurophylline) and Mercuhydrin Sodium (*5*) as diuretics.

Blue tetrazolium (BT) and neotetrazolium (NT) were employed as histochemical indicators for the enzyme. The incubation mixtures were prepared according to Seligman and Rutenburg (*6*).

Mercurophylline in subcutaneous doses of 15 to 30 mg of Hg/kg of weight caused, within 24 hr, an almost complete inhibition of succinic dehydrogenase in the thick portions of Henle's loops, thus confirming our earlier results. This was seen both in BT- and NT-preparations (Fig. 1). The activity of the proximal convoluted tubules, especially of their straight terminal portions, was also clearly reduced. The distal convoluted tubules retained a little more of their activity.

In contrast, the administration of mercuhydrin in the same doses resulted in a very pronounced inhibition of succinic dehydrogenase in the proximal convoluted tubules, whereas the activity in the distal convoluted tubules, and especially in the thick portions of Henle's loops, was only slightly reduced, if at all (Fig. 1). These observations are in essential agreement with the results of previous investigators (*2, 3*).

Fig. 1. BT-preparations on the left, NT-preparations on the right. Top, controls; middle, inhibition pattern by mercurophylline, 15 mg of Hg/kg of weight; bottom, inhibition pattern by mercuhydrin, 15 mg of Hg/kg of weight. Section thickness, 40μ.

Both of these diuretics contain the same amounts of mercury and theophylline. Therefore, the different organic components of these mercurials seem to be responsible for the difference in the enzymatic inhibition, probably by modifying their mode of excretion.

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References and Notes

1. K. K. Mustakallio and A. Telkkä, *Science* **118**, 320 (1953).
2. M. Wachstein and E. Meisel, *ibid.* **119**, 100 (1954).
3. E. G. Rennels and A. Ruskin, *Proc. Soc. Exptl. Biol. Med.* **85**, 309 (1954).
4. Kindly supplied by Messrs. Medica, Helsinki.
5. Kindly supplied by Lakeside Laboratories, Inc., Milwaukee, Wis.
6. A. M. Seligman and A. M. Rutenburg, *Science* **113**, 317 (1951).

8 November 1954.