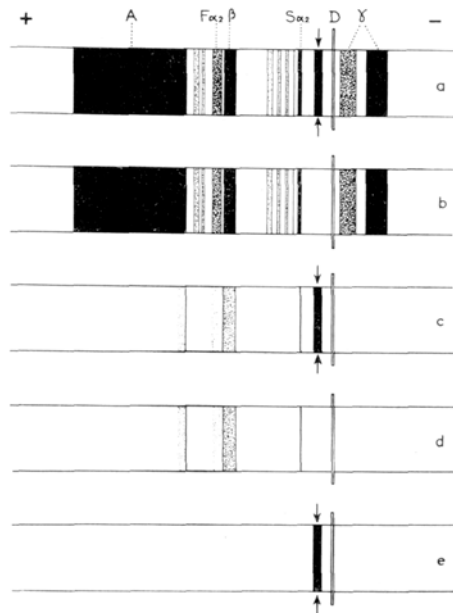


mann 1,2 g, H<sub>2</sub>O distillée 400 ml, Ethanol 600 ml, NaOH 2 ml).

La décoloration du fond est obtenue en laissant le lipodigamme dans de l'alcool à 60° pendant plusieurs jours (8 jours suffisent en général). Ce n'est en effet qu'au bout d'un temps de décoloration suffisamment prolongé que l'on voit apparaître certaines bandes de faible intensité.

**Résultats.** 1°. *Sérum humain total.* Celui-ci présente après coloration au Noir Soudan une zone extrêmement colorée située entre le dépôt du sérum et les  $\alpha_2$  globulines lentes (S  $\alpha_2$ ). Cette zone est également visible sur le protéinogramme coloré à l'Amidoschwarz (Fig. 1a et 1c).



a Sérum humain normal; b Sérum débarrassé sélectivement des bêta-lipoprotéines; c Sérum humain normal; d Sérum débarrassé sélectivement des bêta-lipoprotéines; e bêta-lipoprotéines précipitées par le Sulfate de Dextrane, en présence de Chlorure de Calcium.

a et b coloration à l'Amidoschwarz; c, d et e coloration au Noir Soudan.

D'autres bandes colorées par le Noir Soudan sont visibles mais présentent une intensité colorée beaucoup plus faible, en particulier:

- une très fine bande correspondant aux  $\alpha_2$ ,
- une correspondant aux globulines  $\beta$ ,
- une migrant légèrement en avant de ces globulines,

enfin, le bord de la tache des albumines est coloré, ce qui pourrait correspondre à la présence, soit d'une lipo-albumine, soit des  $\alpha_1$  lipoprotéines mal séparées des albumines par l'électrophorèse en gel d'amidon.

2°. *Sérum débarrassé de bêta-lipoprotéines.* Après coloration au Noir Soudan, toutes les bandes décrites dans le sérum normal sont visibles à l'exception de la fraction lipoprotéique la plus importante située entre le point de dépôt et les globulines  $\alpha_2$  lentes (Fig. 1d).

Sur le protéinogramme, on note la disparition de cette bande située entre le point de dépôt et les globulines  $\alpha_2$  lentes (Fig. 1b).

3°. *Les bêta-lipoprotéines* se manifestent après coloration au Noir Soudan sous forme d'une fraction unique située entre le point de dépôt et les globulines  $\alpha_2$  lentes (Fig. 1e).

**Conclusion.** Après électrophorèse en gel d'amidon la fraction la plus importante des lipoprotéines sériques

humaines migre entre le point de dépôt et les globulines  $\alpha_2$  lentes.

Il s'agit de la fraction qui précipite sélectivement par le Sulfate de Dextrane en présence de Chlorure de Calcium et qui migre sur papier comme les bêta-lipoprotéines.

L'étude comparée du sérum normal et du sérum sélectivement débarrassé des lipoprotéines de faible densité permet de conclure que cette fraction est caractérisée par une bande unique visible sur le protéinogramme.

Sa faible mobilité en électrophorèse en gel d'amidon peut être expliquée par son poids moléculaire élevé.

J. M. FINE et M. BURSTEIN

Centre national de transfusion sanguine Paris, 20 juin 1958.

### Summary

The electrophoretic pattern of human serum lipoproteins after migration in starch gel is different from that obtained by other zone electrophoresis techniques. The most important component which migrates between the slot and slow alpha - 2 globulins may be identified as the component being precipitated by dextran Sulfate and calcium chloride. The other components have the same mobility as either beta or alpha globulins.

## Changes in the Nucleic Acid Content in the Rat During Postembryonal Development

The biochemical changes taking place in the organism in the course of embryonal development are not finished with the birth of the animal. The biochemical composition of the body in the postembryonal period of development is significantly altered by the endocrine organs completing their development in this period. Among other endocrine organs, the hypophysis begins its complete functioning only after birth.

HELLER<sup>1</sup> found in the hypophysis of mature rats 10 times more adiuretin than in the hypophysis of newborn rats, values being corrected for the body weight. The newborn rats are not able to concentrate urine in the same degree during the dehydration as the full developed rats. SCHREIBER<sup>2</sup> followed, in newborn rats, the dependence between the evolution of postembryonal water metabolism and the postembryonal functional evolution of the eye. He observed that in the course of postembryonal evolution the procentual share of dry matter in the total weight of the organism of rats which already see, grows faster than in the course of postembryonal evolution of rats before the opening of the eyes. According to SCHREIBER, it is the consequence of completed evolution of neurohypophysis in the period of the accomplishment of the functional development of the eye. DVOŘÁK<sup>3</sup>, who studied the weight of total lipoids and of cholesterol in rats during the course of postembryonal evolution, observed that the procentual content of cholesterol and of total lipoids increases with the increasing weight quicker in the blind rats than in rats which already see. He explains his results by increased utilization of lipoids related to a new state of the hypophysis development.

<sup>1</sup> H. HELLER, J. Physiol. 106, 28 (1947).

<sup>2</sup> V. SCHREIBER, Časopis lékařů českých 89, 549 (1950).

<sup>3</sup> Z. DVOŘÁK, Nature 171, 432 (1953).

In the present work, the content of desoxyribonucleic acid (DNA) and of ribonucleic acid (RNA) in albino rats was followed during the first part of postembryonal evolution. Special attention was concentrated on the period when the rats begin to see.

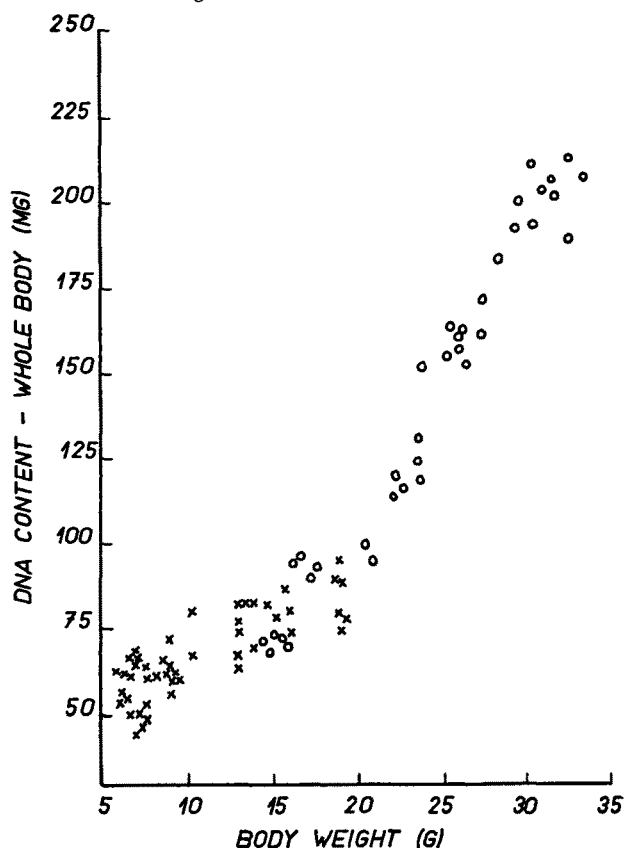


Fig. 1.—Relation of the whole-body DNA content to the body-weight of newborn rats.

× rats which could not see; ○ rats which could see.

88 rats from 19 litters were killed in days following the first day after birth until the 29th day of age. The day when the animals began to see was registered. That happened normally about the thirteenth day after birth. The whole animals were minced in a Waring Blendor. The homogenate was fractionated, using SCHNEIDER's fractionation procedure<sup>4</sup>. The DNA was determined with the DISCHE's reaction<sup>5</sup> and the RNA was determined using the modified Mejbaum's reaction<sup>6</sup>.

It is evident from these figures that the total body amount of DNA and of RNA increases more quickly with increasing weight in rats which already see than in the rats which are still blind. In rats which had just begun to see, the trend of evolution of body's nucleic acids content was changed. From the embryonal state to maturity, the procentual content of nucleic acids in the body continually decreased. On the third day following birth, the rat contained DNA in amounts equal to 0.78%, and RNA in amounts equal to 0.73% of its body weight. The analytical data for the 14-days old, blind rats are 0.50% for DNA and 0.54% for RNA; for a mature six months old rat 0.26% for DNA and 0.41% for RNA. In rats just beginning to see, the increase of nucleic acids is more rapid than in rats before the opening of the eyes. The procen-

tual content of the nucleic acids in the total body weight temporarily increased. This increase is most evident between the fifth and eleventh day after the rats begin to see. At this time the rat's body contains 0.62% of its weight as DNA and 0.68% of its weight as RNA on the average. From the eleventh day, the procentual content

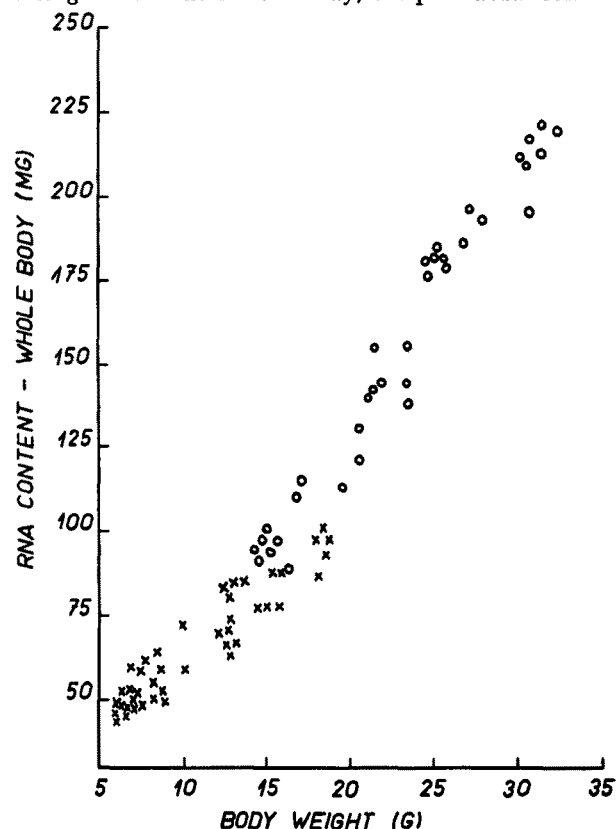


Fig. 2.—Relation of the whole-body RNA content to the body-weight of newborn rats.

× rats which could not see; ○ rats which could see.

of nucleic acids in the body begins to decrease again. This temporary interruption of the trend for procentual decrease of nucleic acids content of the body during ontogenesis, can be explained by the action of impulses from the freshly matured visual organ on the diencephalo-pituitary system. It is well-known that the optical impulses stimulate the secretion of adrenocorticotrophic and growth hormones<sup>7</sup> and exercise an influence on the secretion of anteriopituitary hormones<sup>8</sup>. As is known, the pituitary hormones affect the concentration and metabolism of nucleic acids in different organs. The team group of LI<sup>9</sup> and of DI STEFANO<sup>10</sup> found that hypophysectomy diminishes the RNA content in organs, and this effect of hypophysectomy could be reversed by the growth hormone. REID<sup>11</sup>, who performed nucleic acids analyses of various fractions of rat liver cytoplasm, has shown characteristic changes in nucleic acids content as well as in the activity of nucleic acids metabolising enzymes<sup>12</sup> for every cytoplasm fraction following hypophysectomy. The more rapid increase of total DNA and RNA amount with

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<sup>8</sup> D. HOFMAN-CREDNER, *Arch. int. Pharmacodyn.* 91, 241 (1952).

<sup>9</sup> I. GESCHWIND, C. H. LI, and H. M. EVANS, *Arch. Biochem.* 28, 73 (1950).

<sup>10</sup> H. S. DI STEFANO, A. D. BASS, H. F. DIERMEIER, and J. TEPERMAN, *Endocrinology* 51, 386 (1952).

<sup>11</sup> E. REID, *Nature* 175, 461 (1955).

<sup>12</sup> E. REID and B. M. STEVENS, *Biochem. J.* 68, 367 (1958).

<sup>4</sup> W. C. SCHNEIDER, *J. biol. Chem.* 161, 293 (1945).

<sup>5</sup> Z. DISCHE, *Mikrochemie* 8, 4 (1930).

<sup>6</sup> L. MASSART and J. HOSTE, *Biochem. biophys. Acta* 1, 83 (1947).

increasing body weight in the rats which could see in comparison with blind rats, may be related to the excretion of pituitary hormones. The findings<sup>3</sup> of smaller increase of lipoids and cholesterol and quicker increase of dry matter in the body of the rats which can see compared with blind rats, can be explained by the more intensive utilization of lipoids as energy donors for the synthesis of proteins stimulated by growth and other pituitary hormones, on the basis of elevated nucleic acids content. This greater increase of nucleic acids in rats beginning to see may be related to the completion of functional evolution of the eyes and to their action on the diencephalo-pituitary system. During postembryonal evolution, the influence of elevated secretion of pituitary hormones returned to normal limits and the procentual content of nucleic acids in the organism continued to decrease in agreement with the trend of ontogenetical evolution of the nucleic acids content in the rat body.

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*Oncological Research Institute, Bratislava and Oncological Institute, Brno (Czechoslovakia), August 4, 1958.*

#### *Zusammenfassung*

Bei Ratten wurde der Gehalt an Desoxyribonukleinsäure und Ribonukleinsäure von der Geburt bis zum 29. Lebenstag verfolgt. Mit Beginn des Sehens erhöht sich der gesamte Nukleinsäuregehalt des Organismus; vorübergehend zeigt sich auch ein prozentualer Anstieg. Diese Erhöhung des Nukleinsäuregehaltes wird als Folge der vermehrten Sekretion der durch den Lichtreiz stimulierten Hypophyse erklärt.

### **Influence of Oxidative Phosphorylation Inhibitors on the Histamine Release in the Anaphylactic Reaction *in vitro***

Several recent experiments have indicated that metabolic factors are closely involved in the mechanism of histamine liberation during the anaphylactic reaction. The importance of the metabolic factors could be demonstrated by employing specific enzymatic inhibitors<sup>1</sup>, or studying the influence of metabolites consumed during the cellular work<sup>2</sup>.

Using the latter method, it could be demonstrated that some metabolites, such as succinate,  $\alpha$ -keto-glutarate, acetate<sup>3</sup>, enhance the amount of histamine released from sensitized guinea-pig lung tissue by the anaphylactic reaction *in vitro*.

MONGAR and SCHILD<sup>4</sup>, studying the effect of a series of enzymatic inhibitors, showed that histamine release can be inhibited by a large number of them, and that the substances which inhibited the release of histamine in anaphylaxis also inhibited oxygen consumption. MONGAR and SCHILD concluded that the anaphylactic reaction requires a functioning cell while admitting that the experiments provided little evidence on the precise nature of the reactions involved.

It seemed important to study the influence of some potent inhibitors of the oxidative phosphorylation on the anaphylactic reaction, since we showed that there is an increase in the oxygen uptake by the tissue<sup>5</sup> during the anaphylactic reaction, indicating an increase in the cellular work.

For the experiments, guinea-pigs were sensitized with horse sera previously treated by potassium alum, according to the Caulfield method. Sensitization of the animals was assayed by the Schultz-Dale reaction on a piece of the ileum. The lungs of sensitized animals were sliced by hand with a sharp razor and the slices transferred to the Ringer Barron medium of the main chamber of Warburg flasks. Sodium succinate, and the enzymatic inhibitor at the required concentration, were kept in the side-bulb of the flasks and tipped into the main chamber after thermal equilibration, or incubated together with the tissue. Antigen was added 30–150 min after incubation and left a further period of 30 min for the histamine liberation. Oxygen uptake was measured every 30 min, and calculated as mm<sup>3</sup>/mg dry weight/h. Histamine was assayed in the supernatant fluid by the guinea-pig ileum method and calculated in  $\mu$ g of histamine dihydrochloride by g wet weight. As inhibitors of the oxidative phosphorylation, 2,4-dinitrophenol, pentachlorophenol, and sodium azide were investigated and the results are given in Table I.

All the three substances assayed caused an effective inhibition of the histamine release. 2,4-dinitrophenol at the concentration of  $10^{-4}$  M, known as uncoupling phosphorylation from cellular oxidation<sup>6</sup>, almost completely inhibited the liberation of histamine, leaving the oxygen uptake normal or even increased. Pentachlorophenol<sup>7</sup> and sodium azide<sup>8</sup> which are known to act like dinitrophenol, prevented significantly the histamine release by the antigen. Decrease in the  $Q_{O_2}$  observed in the experiments with the azide cannot explain the lowering in the histamine released. Using low tensions of oxygen (10%  $O_2$  and 90%  $N_2$ ), a decrease of the  $Q_{O_2}$  of around 50% can be obtained without decrease in the histamine released<sup>9</sup>.

In another series of experiments, the influence of 2,4-dinitrophenol on the histamine released by the lungs was investigated in a medium containing succinate and  $\alpha$ -keto-glutarate. The results of Table II show the strong inhibitory action of 2,4-dinitrophenol, both on the additional histamine released by the effect of substrates, and by the antigen alone.

These experiments give suggestive evidence that the histamine released during the anaphylactic reaction needs energy-rich phosphorylated compounds. The experiments with succinate and other metabolites of the Krebs cycle can be explained by supposing that the oxidation of these substrates led to an increase in available phosphorylated compounds<sup>10</sup>.

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