SPECIAL ARTICLES

EGG-WHITE INJURY AS THE RESULT OF NONABSORPTION OR INACTIVATION OF BIOTIN[1]

EGG-white injury is produced in animals fed a diet that contains a large proportion of fresh or commercial egg white and is devoid of a specific protective factor (vitamin H). The recent identification[2] of vitamin H with biotin made the experimental analysis of the syndrome of egg-white injury easier and less time-consuming through the use of the simple yeast growth method for quantitative determination of biotin (vitamin H). In contrast to the laborious animal assays which require a long preparatory period of from 6 to 10 weeks with a subsequent test period of 4 weeks,[3] the biotin test can be performed in less than 24 hours.[4]

By the microbiological method it has been demonstrated[5] that the tissues of chicks on a diet causing egg-white injury were deficient in biotin (vitamin H) despite the abundance of this vitamin in the diet. In continuation of these studies experimental evidence has recently been presented[6] to show that commercial or fresh egg white is capable of inactivating biotin *in vitro,* owing probably to the formation of a fairly stable compound of biotin with a special constituent of egg white. This particular fraction of egg white in further purification has exhibited albumin-like properties. Following a proposal of the Texas group it has been tentatively called "avidalbumin" (literally, hungry albumin).

The next problem, namely, whether the biotin-binding capacity of the avidalbumin fraction may also explain its apparently "toxic" effect when it is fed to animals, has been studied in direct trials on rats.

For this special purpose there has been utilized the well-known fact that egg white loses its "toxic" properties when it is subjected to continued and intensive treatment with heat.[7] In preliminary experiments it has been found that rats with well-developed egg-white injury improve remarkably within 2 weeks after cooked dried egg white has been substituted for the original commercial egg white in the experimental diet.[8] The skin clears up and growth is resumed to a considerable degree.

In the principal experiments avidalbumin concentrates have been thoroughly mixed with pulverized cooked dried egg white in an amount which corresponded, on the basis of biotin-binding capacity, to the equivalent amount of fresh egg white. For instance, in one group of experiments 231 mg of concentrate represented 100 gm of dried fresh egg white and was consequently added to 100 gm of dried cooked (inactivated) egg white. At the same time control animals were kept on a diet which without any further change contained the same proportion (30 per cent.) of cooked egg white but no added avidalbumin. Owing to slight fluctuation in purity, the concentration of avidalbumin preparation in the diet was not a constant figure but varied in the experiments between 0.03 and 0.07 per cent.

The results obtained speak unequivocally in favor of the assumption that avidalbumin has to be considered the "toxic" constituent of egg white, as it causes the specific syndrome of egg-white injury when it is included in the diet fed to rats. When the diet containing cooked (inactivated) egg white and avidalbumin was fed to rats suffering from egg-white injury during the whole of the experimental period of 2 weeks not the slightest improvement was observed. On the contrary, without exception, the condition of the rats deteriorated considerably, proving the "toxic" effect of the avidalbumin fraction. In contrast, rats fed the same diet minus avidalbumin, with or without a preceding period in which cooked egg white plus avidalbumin was fed with the regular deteriorating effect, have shown considerable improvement in 2 weeks.

SELECTED EXAMPLES

Rat No. 6096. Period I (2 weeks) with avidalbumin; loss in weight 4 gm; severe egg-white injury. Period II (2 weeks) without avidalbumin; gain in weight 32 gm; egg-white injury improved.

Rat No. 6097. Period I (2 weeks) with avidalbumin; loss in weight 11 gm; severe disease. Period II (2 weeks) without avidalbumin; gain in weight 35 gm; almost cured.

Similar results were obtained in a group of animals fed a diet that contained only two thirds of the equivalent amount of avidalbumin. This fact proves the high activity of the avidalbumin fraction.

The experiments here reported prove conclusively that the "toxicity" of egg white can be attributed to its avidalbumin fraction. On the other hand, it is known

[1] In this cooperative research the biotin binding protein was prepared and furnished by the Texas group, and the Cleveland group was responsible for the animal experiments.

[2] P. György, D. B. Melville, D. Burk and V. du Vigneaud, SCIENCE, 91: 243, 1940; V. du Vigneaud, D. B. Melville, P. György and C. S. Rose, SCIENCE, 92: 62, 1940; P. György, C. S. Rose, K. Hofmann, D. B. Melville and V. du Vigneaud, SCIENCE, 92: 609, 1940.

[3] P. György, *Jour. Biol. Chem.*, 131: 733, 1939.

[4] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

[5] R. E. Eakin, W. A. McKinley and R. J. Williams, SCIENCE, 92: 224, 1940.

[6] R. E. Eakin, E. E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 136: 801, 1940.

[7] H. T. Parsons and E. Kelly, *Am. Jour. Physiol.*, 104: 150, 1933.

[8] György (3), p. 737.

that this fraction is able to neutralize biotin *in vitro* and that this action is due to the formation of a fairly stable compound. Thus egg-white injury is explained by the unavailability of biotin because of its fixation to the avidalbumin. It can be assumed that in the presence of avidalbumin biotin is not even absorbed from the intestinal tract and is excreted with the feces. This would then also throw light on the question why biotin should exert a potency three to five times higher in parenteral administration than in oral.[9]

From one point of view egg-white injury is a secondary deficiency disease and belongs in the same group as deficiency manifestations in sprue, for instance, or in other chronic diarrheic conditions. It also bears some similarity to the possible effect of long-continued medication of mineral oil on the vitamin A (carotene)[10] and vitamin D[11] reserves of the body, with the important distinction, among others, that in contrast to avidalbumin mineral oil is not a regular food constituent.

The mechanism of egg-white injury appears to represent a hitherto unknown principle in the production of disease.[12]

PAUL GYÖRGY
CATHARINE S. ROSE
THE BABIES AND CHILDRENS HOSPITAL,
AND THE DEPARTMENT OF PEDIATRICS,
SCHOOL OF MEDICINE, WESTERN
RESERVE UNIVERSITY, CLEVELAND

ROBERT E. EAKIN
ESMOND E. SNELL
ROGER J. WILLIAMS
THE DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF TEXAS, AUSTIN

THE EXCRETION OF INJECTED HEPARIN IN THE URINE OF MICE AND DOGS

THERE is some controversy in the literature as to whether heparin is excreted in the urine following its intravenous injection. Howell and McDonald[1] and Wilander[2] reported that heparin is excreted by the kidney. Jaques[3] on the contrary recently reported that heparin does not appear in the urine. We, therefore, tested the urine of heparinized mice and dogs for the presence of heparin.

METHOD

According to Lison[4] the purple color obtained with toluidine blue is specific only for sulfuric acid esters of high molecular weight. Jorpes and Bergström[5] considered heparin to be a mucoitin polysulfuric ester and Jorpes[6] tested this metachromatic reaction on heparin solution. He found that the color with heparin is about one hundred times more intense than with chondroitin sulfuric acid.[7] We confirmed his observations and moreover found that the heparin of the Connaught Laboratories (110 units per mgm) was about 1,100 times more intense than chondroitin (Wilson and Company). The method is very simple: 0.5 cc of toluidine blue in distilled water 1:5000 is added to 0.5 cc of diluted or undiluted urine. If heparin is present a purple color results. A precipitate is gradually formed and after 30 minutes its density is compared with a series of standard solutions containing 1 to 6 units of heparin to the same amount of normal urine. Two hundred to 1,000 units of heparin per 20 grams weight were injected subcutaneously into 11 mice. Three to 6 hours after the injection the urine showed a purple color with toluidine blue. One hundred and 200 units of heparin per kgm of body weight were injected intravenously into 12 dogs. Samples of urine were obtained before heparinization and one hour after the heparin was injected.

RESULTS

Normal urine never showed the purple reaction whereas the urine of the heparinized animals always developed a purple color. The estimated number of units of heparin could be computed to the volume of urine obtained by catheterization. It was thus possible to measure in a certain time interval the approximate amount of heparin excreted by the kidneys. As further proof, the urine was also tested for its anticoagulant action upon blood. It was found that urine from heparinized dogs prolonged the coagulation time of blood whereas urine from the same dogs before heparinization did not.

We conclude that heparin injected subcutaneously in

[9] P. György and C. S. Rose, *Proc. Soc. Exp. Biol. and Med.*, 43: 73, 1940.

[10] A. C. Curtis and P. B. Horton, *Am. Jour. Med. Sci.*, 200: 102, 1940; M. T. Burrows and W. K. Farr, *Proc. Soc. Exp. Biol. and Med.*, 24: 719, 1926–27; R. A. Dutcher, J. O. Ely and H. E. Honeywell, *Proc. Soc. Exp. Biol. and Med.*, 24: 953, 1926–27; R. A. Dutcher, P. L. Harris, E. R. Hartzler and N. B. Guerrant, *Jour. Nutrition*, 8: 269, 1934.

[11] M. C. Smith and H. Spector, *Jour. Nutrition*, 20: 19, 1940.

[12] Aqueous solution of tobacco mosaic virus (containing 25 mg per cubic centimeter), kindly furnished by Dr. W. M. Stanley, Rockefeller Institute, Princeton, N. J., has been tested for its biotin-binding capacity. The test showed (1) that the virus did not inactivate biotin in so far as the yeast is concerned and (2) that no appreciable amount of biotin is freed by heating the virus in solution.

[1] W. H. Howell and C. H. McDonald, *Bull. Johns Hopkins Hosp.*, 46: 365, 1930.

[2] O. Wilander, *Skandinav. Arch. f. Physiol.*, 81: Suppl. xv, 1939.

[3] L. B. Jaques, *Am. Jour. Physiol.*, 125: 98, 1939.

[4] L. Lison, *Compt. rend. Soc. de biol.*, 118: 821, 1935; *Arch. de Biol.*, 46: 599, 1935; *Bull. Soc. chim. biol.*, 18: 225, 1936.

[5] E. Jorpes and S. Bergström, *Jour. Biol. Chem.*, 118: 447, 1937.

[6] E. Jorpes, *Acta med. Scandinav.*, 88: 427, 1936.

[7] E. Jorpes, *Acta med. Scandinav. Suppl.*, LXXXIX: 139, 1938.