pronounced response of the adrenals. The stimulating effect on the insulin-releasing mechanism becomes evident only after the latter effect has been abolished. A similar interpretation was given by Gellhorn and his collaborators[4] to results of their interesting study on the mutual relationship of the vago-insulin and sympatho-adrenal systems under conditions of central excitation induced by anoxia and by metrazol. These investigators confirmed the hypoglycemic response to anoxia in adrenalectomized rats and found in addition that denervation of the adrenals had an effect similar to that of adrenalectomy. Metrazol, like anoxia, produced a hyperglycemia in normal but a hypoglycemia in adrenalectomized rats. They then found that intraäbdominal section of the vagi of adrenalectomized rats abolished the hypoglycemic response to either anoxic anoxia or metrazol. The effect of pancreatectomy in the present experiments was practically identical with that of vagotomy in their study.

*Conclusions.* Since pancreatectomy has been shown to abolish the hypoglycemic response of the adrenalectomized animals to anoxic anoxia, it may be concluded with Gellhorn that anoxia normally stimulates the parasympathetic (insulin response) as well as the sympathetic (adrenal response) system.

## 13544

## Distribution of Biotin and Avidin in Hen's Egg.

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Identification, with biotin,[1] of the curative factor (vitamin H) for egg-white injury in rats, and the recognition that avidin,[2] a protein-like constituent of egg white, is the injury-producing principle[3] of raw egg white raised the question of the distribution of

[4] Feldman, J., Cortell, R., and Gellhorn, E., *Am. J. Physiol.*, 1940, **131**, 281.

[1] György, P., Melville, D. B., Burk, D., and du Vigneaud, V., *Science*, 1940, **91**, 243; du Vigneaud, V., Melville, D. B., György, P., and Rose, C. S., *Science*, 1940, **92**, 62.

[2] Eakin, R. E., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1940, **136**, 801; 1941, **140**, 535.

[3] György, P., Rose, C. S., Eakin, R. E., Snell, E. E., and Williams, R. J., *Science*, 1941, **93**, 477.

these substances in whole egg. The search for an answer is especially appropriate when it is recalled that in the investigations of Kögl and Tönnis[4] biotin was isolated from egg yolk, whereas, on the other hand, raw egg white is the only known source of avidin.

In the main, the problem is two-fold: 1. Does the whole egg contain a surplus of avidin or of biotin? 2. How can free biotin in egg yolk and excess of avidin in egg white exist simultaneously in the intact egg?

*Experimental Method.* Free biotin was determined by the yeast growth method.[5] Biotin bound to avidin (AB) was freed by steaming and then determined microbiologically as biotin. The concentration of avidin in excess is expressed in terms of its biotin-binding capacity.[2] The values obtained for free biotin in egg yolk were controlled by experiments on rats suffering from egg-white injury which were fed a diet containing a large proportion of egg white.[6] In the experiments with egg yolk special care was taken to avoid admixture of egg white (avidin).

*Experiments.* Group 1. Quantitative determinations of biotin and avidin in whole egg in which egg white and egg yolk were thoroughly mixed invariably showed an excess of avidin, as illustrated by two examples:

| | Avidin total Free (A) + bound (AB) μg/cc | Avidin in form of biotin complex (AB) μg/cc | Excess of free avidin (A) μg/cc |
|---|---|---|---|
| (*a*) | .50 | .19 | .31 |
| (*b*) | .54 | .30 | .24 |

These results were confirmed indirectly by experiments in which egg white, egg yolk (of one egg) and mixtures of yolk and white in the proportion found in the whole egg were analyzed, as shown by the following values:

| | | Biotin (free *plus* bound) | | | Avidin (free *plus* bound) | | |
|---|---|---|---|---|---|---|---|
| | | Found | | | Found | | |
| | Vol., cc | μg/cc | μg/total vol. | Calculated μg/total vol. | μg/cc | μg/total vol. | Calculated μg/total vol. |
| White | 27 | .15 | 4.1 | — | .70 | 19.0 | — |
| Yolk | 14 | .53 | 7.4 | — | .0 | .0 | — |
| Whole egg | 41 | .30 | 12.3 | 11.5 | — | 22.0 | 19.0 |

Group 2. In Group 1 the biotin values for egg yolk were obtained

[4] Kögl, F., and Tönnis, B., *Z. Physiol. Chem.*, 1936, **242**, 43.

[5] Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175; *Univ. Texas Pub.* No. 4137, 1941, p. 18.

[6] György, P., *J. Biol. Chem.*, 1939, **131**, 733.

by using a suspension of egg yolk in saline solution without or with steaming. The possible objection that this method may leave part of the biotin content of the egg yolk unaccounted for has been investigated in special experiments. Furthermore, the egg yolk was subjected to treatment of various kinds before the quantitative microbiological tests for biotin were carried out. It was hoped that the results obtained might shed light on the form in which biotin occurs in egg yolk. A few examples are summarized in Table I, as illustrative of the results obtained.

The tentative conclusion may be drawn that suspension of egg yolk in cold saline solution gives values for biotin which are, as a rule, either equal to or only moderately lower than the values found in aliquot portions of the same saline suspensions after acid hydrolysis or after steaming. Equally high values were obtained in samples which were "extracted" after steaming with distilled water in varying dilutions. In contrast, the filtrates from suspensions of egg yolk in cold distilled water contained only traces of the biotin found in "hot" distilled water or in saline extracts or in the hydrolysates. Filtering with kieselguhr, as well as precipitation with acetone, diminished the biotin content of the saline extracts. Although the differences obtained in the biotin values in cold and steamed samples of egg yolk "extracts" in distilled water are reminiscent of the behavior of avidin in egg white, the fact that cold "extract" in saline gives values almost as high as those obtained after steaming cannot be reconciled with the assumption of an avidin-like substance in egg yolk. The results summarized in Table I speak rather in favor of the conclusion that biotin is present in egg yolk in combination with a larger molecule, probably a globulin-like constituent of the egg yolk. This compound can be precipitated by cold distilled water, partly also by acetone; it is soluble to a large extent in cold saline and can be split, with simultaneous liberation of biotin, by steaming for a short time. Biotin is available for yeast cells even in the form of the high molecular protein compound.

Biotin liberated from liver by hydrolysis is freely dialyzable,[7] as is pure biotin itself. If the biotin of egg yolk should be found to be present in the form of a high molecular compound, it should be undialyzable. That it is undialyzable has been proved unequivocally in experiments summarized in Table II and was also indicated by the recent investigations of Williams and his collaborators.[8]

Since this biotin of egg yolk is available for yeast cells, the

[7] Birch, T. W., and György, P., *J. Biol. Chem.*, 1939, **131**, 761.

[8] Thompson, R. C., Eakin, R. E., and Williams, R. J., *Science*, 1941, **94**, 590.

TABLE I.
Content of Biotin in 3 Samples of Egg Yolk Subjected to Various Treatments.

| | Treatment | Biotin ($\mu$g/cc) A* | B* | C* |
|---|---|---|---|---|
| Saline dilution (cold) | 1 cc egg yolk to 100 cc with saline: | | | |
| | (*a*) passed through Whatman filter paper No. 42† | .34 | .60‡ .65‡ | .32 |
| | (*b*) filtered with kieselguhr | | .02‡ .06‡ | |
| Saline dil. (steamed) | 1 cc egg yolk to 100 cc with saline, steamed 15 min: | | | |
| | (*a*) passed through filter paper No. 42† | .41 | .60‡ .66‡ | .59 |
| | (*b*) filtered with kieselguhr | — | — | .54 |
| Distilled water dil. (cold) | 1 cc egg yolk to 10 cc with distilled $H_2O$: | | | |
| | (*a*) passed through filter paper No. 42 until clear as possible | — | .009‡ .02‡ | .03 |
| | (*b*) filtered with kieselguhr | — | — | .015 |
| | 1 cc egg yolk to 100 cc with distilled $H_2O$, passed through filter paper No. 42: filtrate clear but test for protein with sulfosalicylic acid positive | .037 | .026 | — |
| | 1 cc egg yolk to 1000 cc with distilled $H_2O$, passed through filter paper No. 42: test with sulfosalicylic acid positive | .032 | .06 | — |
| Distilled water dil. (steamed) | 1 cc egg yolk to 10 cc with distilled $H_2O$, steamed 15 min: | | | |
| | (*a*) passed through filter paper No. 42 until clear as possible | — | — | .52 |
| | (*b*) filtered with kieselguhr | — | — | .41 |
| | 1 cc egg yolk to 100 cc with distilled $H_2O$, steamed 15 min: | | | |
| | (*a*) passed through filter paper No. 42 until clear as possible | .36 | — | — |
| | (*b*) passed through filter paper No. 42† | .41 | — | — |
| | (*c*) filtered with kieselguhr | .38 | — | .52 |
| Hydrolyzed sample | 1 cc egg yolk to 10 cc in 6*N* $H_2SO_4$, autoclaved 1 hr neutralized, filtered through paper No. 42† | — — — | .63‡ .60‡ .65‡ | — — — |
| Acetone extract | 1 cc egg yolk to 10 cc with saline, precipitated with 4 times vol. of acetone, diluted with water and fat removed by means of ether (free from peroxides), water layer evaporated to dryness and tested for biotin | — | .26 | .21 |

*Each vertical column represents a different sample of egg yolk.
†Before filtering, dilutions were adjusted to pH 3.5.
‡Same egg yolk, tested on different days.

question arose whether it is equally available for rats in the cure of egg-white injury. It was found that rats were completely cured of egg-white injury when they were given daily injections of suspensions of egg yolk in saline which contained amounts of biotin as

TABLE II.
Dialyzability of Biotin in Different Samples of Egg Yolk.

| | Treatment | Biotin (μg/cc) | | | |
|---|---|---|---|---|---|
| | | B* | C* | D* | E* |
| Saline dilutions (cold) | 1 cc egg yolk to 100 cc with saline, dialyzed 24 hrs in cold room, steamed 15 min, filtered†: | | | | |
| | Control‡ | .68 | .62 | .69 | — |
| | Dialyzed sample | .75 | .58 | .55 | — |
| | Biotin added to egg yolk (0.2 μg/cc to B, 0.4 μg/cc to D); 1 cc egg yolk to 100 cc with saline, dialyzed 24 hrs in cold room, steamed 15 min, filtered†: | | | | |
| | Control‡ | .88 | — | 1.1 | — |
| | Dialyzed sample | .53 | — | .66 | — |
| Saline dil. (steamed) | 1 cc egg yolk to 100 cc with saline, steamed 15 min, filtered†, then dialyzed 24 hrs in cold room: | | | | |
| | Control‡ | — | — | .69 | — |
| | Dialyzed sample | — | — | .035 | — |
| Distilled water dilution (cold) | 1 cc egg yolk to 100 cc with distilled water, filtered,† dialyzed 24 hr in cold room, steamed 15 min | | | | |
| | Control‡ | — | — | — | .023 |
| | Dialyzed sample | — | — | — | .015 |

*Each vertical column represents a different sample of egg yolk. For other data on samples B and C, see Table I.

†Before being passed through Whatman filter paper No. 42, these dilutions were adjusted to pH 3.5.

‡Control samples were kept at the same temperature and for the same length of time as the dialyzed samples before being assayed.

low as 0.2, 0.1, and even 0.05 μg, as determined by the yeast growth method. The smallest amount (0.05 μg) proved to be approximately the "rat day dose."[9] Thus biotin in egg yolk, although bound in a high molecular, undialyzable form, is, physiologically at least, as active as pure biotin.

*Conclusions.* 1. Whole egg contains an excess of avidin, as the amount of biotin in the whole yolk is unable to neutralize the amount of avidin in the white of the same egg. 2. Biotin in egg yolk is present in a high molecular, undialyzable form which is physiologically active in yeast growth tests or in tests made on rats with egg-white injury.

[9] György, P., Rose, C. S., Hofmann, K., Melville, D. B., and du Vigneaud, V., *Science*, 1940, **92**, 609; du Vigneaud, V., Hofmann, K., Melville, D. B., and György, P., *J. Biol. Chem.*, 1941, **140**, 643.