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The Nature of the Respiratory Supplement.

K. SALOMON AND L. MICHAELIS.

*From the Laboratories of The Rockefeller Institute for Medical Research,
New York.*

When the oxygen consumption of rabbit erythrocytes and that of saline extracts of rat livers (or various other organs), are measured separately, and compared with what happens in a mixture of both, the oxygen consumption is much higher in the mixture than the sum of the oxygen consumptions in the 2 separate systems. This has been shown by the authors previously¹; the effect of the liver extract has been confirmed by Zeile and Euler.²

This paper presents the attempts to elucidate the nature of this reaction. For brevity's sake the active principle of the liver extract will be designated as the respiratory supplement.

1. The supplement deteriorates and may even be destroyed by various mechanical treatments of the liver extract. Extended shaking, in presence or in absence of oxygen damages the supplement. When the extract is centrifuged the supplement activity both of the sediment and of the supernatant fluid is diminished and by mixing the 2 fractions is not restored to its original strength.

2. Several attempts were made to isolate the supplement by adsorption. Sand, Kaolin, Kieselgur adsorb the supplement to only a small extent, according to the amount applied, but never completely. Colloidal iron hydroxyd adsorbed the supplement completely in several experiments, in which the adsorbent was used in an amount sufficient to remove the proteins. Colloidal aluminum hydroxyd removed it completely when applied in so small an amount as to remove only a small part of the proteins. No attempt to re-

¹ Michaelis, L., and Salomon, K., *J. Gen. Physiol.*, 1930, **13**, 683.

² Zeile, K., and v. Euler, H., *Hoppe-Seyler's Z. f. physiol. Chem.*, 1931, **195**, 35.

cover the supplement by elution with N/20 ammonia was successful.

3. Heating to 56° diminishes the supplement considerably though there is no critical temperature for this effect. The inactivation by heat seems to run parallel to the formation of a coarse turbidity or flocculation in the previously slight and homogeneous extract. Freezing did not impair the effect of the supplement at all.

4. The liver extract does not seem to be able to convert hemoglobin to methemoglobin, at least not to a degree detectable with certainty.

5. The supplementary effect of methylene blue (instead of liver extract) first observed by Harrop³ and Barron⁴ and investigated subsequently by Barron,⁵ O. Warburg⁶ and Wendel⁷ is manifested only when the erythrocytes are intact and vanishes in experiments with hemolyzed erythrocytes. This is sometimes the case with liver supplement also as described in our previous paper. However, this phenomenon is not as regular with the liver supplement as with methylene blue. Sometimes the previous laking of the erythrocytes by repeated freezing and thawing, which destroys the effect of methylene blue, has only a slight damaging effect upon the action of the liver supplement.

It is, therefore, evident that the supplement is not simply comparable in nature and action with methylene blue. It has some intrinsic connection with what we may call the colloidal state, or a remainder of some kind of "structure", in the liver extract.

Press juice of liver obtained with Kleinmann's hydraulic micro-press and diluted to a volume comparable to that of the usual extract, acts like the extract.

It is noteworthy, furthermore, that the sum of the oxygen consumption of the separated systems (the liver extract and the blood corpuscles) has no noticeable correlation with the oxygen consumption of the mixture. It may happen that the oxygen consumption of each of the components is negligibly small and the oxygen consumption in the mixture is very large.

The reproducibility of the original experiment with liver extract and intact erythrocytes, such as described in the previous paper

³ Harrop, G. A., and Barron, E. S. G., *J. Exp. Med.*, 1928, **48**, 207.

⁴ Barron, E. S. G., and Harrop, G. A., *J. Biol. Chem.*, 1928, **79**, 65.

⁵ Barron, E. S., *J. Biol. Chem.*, 1929, **81**, 445.

⁶ Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1930, **227**, 245.

⁷ Wendel, W. B., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 401.

turned out to be very good. Out of about 75 independent experiments there were only 3 in which the oxygen consumption of the mixture was not at least 3 times that of the isolated components; often it was 10 times that.

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The Enterogastric Reflex.

J. EARL THOMAS AND C. J. MOGAN.

From the Department of Physiology, Jefferson Medical College, Philadelphia.

Numerous investigators have reported a delay in gastric evacuation as a result of placing acid, fat, gastric juice, etc., in the duodenum or other parts of the small intestine. The effect has generally been attributed to a reflex (the "Hirsch v. Mering reflex") causing tonic contraction of the pyloric sphincter. However, two studies record inhibition of contractions of the *pars pylorica* following the introduction of acid¹ or fat² into the duodenum. Apparently both investigations were made upon the empty stomach, though Edelmann² observed inhibition of gastric contractions during secretion of gastric juice. There are no data in either report to prove that the phenomenon occurs in the full stomach or has any bearing on the mechanism of gastric evacuation.

We studied the effect of placing various substances in the duodenum while recording graphically the tone changes in the pyloric sphincter by means of the pressure tonometer,³ and the contractions of the *pars pylorica* by means of a balloon placed in the pyloric canal. Observations were made before, during and after feeding. A balloon in the pyloric canal of the empty stomach registers contractions which are indistinguishable graphically from digestive peristalsis and are probably aroused by the presence of the balloon. We do not consider them hunger contractions. Dogs were used, some narcotized, others without anesthesia or narcosis. The latter were provided with gastric and duodenal fistulas fitted with cannulas.

HCl N/10 in the duodenum, inhibits the contractions of the *pars pylorica* in both the full and empty stomach. In the former the

¹ Kirschner, M., and Mangold, E., *Mittell. a. d. Grenzgeb. d. Med. u. Chirurg.*, 1911, **23**, 446.

² Edelmann, J., *Jahresbericht f. Tier-Chemie (Malys Jahresb.)*, 1906, **36**, 414.

³ Thomas, J. Earl, *Am. J. Physiol.*, 1929, **88**, 498.