One rat that died after 4 injections of *S. faecalis* showed bacterial emboli in many glomerular and intertubular capillaries throughout both kidneys.

Three injected animals had old calcifying sterile renal infarcts (typical of altitude exposure), and 1 had a recent infarct with a markedly congested border densely infiltrated by polymorphonuclear leucocytes. There were many marginal micro-abscesses and scattered clumps of streptococci, some extending into the infarcted area.

The lungs of many rats that died showed intense congestion and often extensive edema, hemorrhages or pneumonia. Several showed centrolobular necrosis in the liver and a few had multiple abscesses chiefly in the lungs or spleen.

Summary and conclusions. Our findings indicate that bacterial endocarditis may be induced readily in rats exposed 4 hours daily to simulated high altitude (25,000 ft.). Twenty-six of 44 altitude rats, but only 1 of 39 non-altitude controls, developed bacterial endocarditis after intravenous injections of *S. mitis, S. sanguis* and *S. bovis* obtained from human cases of subacute bacterial endocarditis. Endocarditis was found in 20 of 26 altitude rats and in 7 of 14 non-altitude controls injected with *S. faecalis*. The incidence of endocarditis and the character of the lesions in other organs varied with the species of streptococcus used. It is suggested that altitude rats may be used to study the reaction and to test the effectiveness *in vivo* of various therapeutic measures against specific strains of organisms causing bacterial endocarditis in man. It is also suggested that exposure to altitude may be a useful experimental method for rendering resistant species of animals susceptible to some diseases other than endocarditis.

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A Fermentable Non-Reducing Substance in Plasma Following Insulin Administration.* (18271)

ARNE N. WICK, DOUGLAS R. DRURY AND EATON M. MACKAY. (With the technical assistance of Richard W. Bancroft, Robert Fitch, Nancy Hillyard, Mary E. Blackwell and Milford E. Dorough)

From the Scripps Metabolic Clinic, La Jolla, Calif., and the Department of Physiology, University of Southern California, Los Angeles.

During the course of a study of the action of insulin on glucose oxidation(1) it was found that insulin administration leads to the appearance of a fermentable (*i.e.* liberates CO_2 under the action of yeast) non-reducing substance in the plasma of rabbits. This report concerns the conditions under which this substance is formed.

Experimental. The general plan of the experiments was as follows. After evisceration(2) the rabbits were injected with a priming dose of a potent preparation of uniformly labeled carbon 14 radioactive glucose. They were then maintained for 8 hours with a normal blood sugar level by the constant injection of a radioactive glucose solution calculated to have the same specific activity as that of the circulating plasma glucose resulting from the priming dose. Two groups of eviscerated animals were used and treated

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1. Wick, A. N., Drury, D. R., Bancroft, R. W. and MacKay, E. M., *J. Biol. Chem.*, Dec., 1950.

2. Drury, D. R., *Am. J. Physiol.*, 1935, v111, 289.

similarly except that one group received insulin and the other not. In addition, experiments were carried out on insulinized normal rabbits. The measurement of C^{14} radioactivity, preparation of the carbon 14 labeled glucose, and other experimental details for the eviscerated rabbits not given here has been reported elsewhere(1).

The glucose concentration of each plasma sample was determined separately by two independent groups of workers. In the one laboratory the method described by Somogyi (3) was employed. The balance of plasma sample was then frozen in dry ice and sent to the other laboratory. Here the Miller and Van Slyke(4) procedure was used in combination with a micro fermentation using Fleischmann's Baker's Yeast in a manner similar to that described by Reinecke(5). In all cases the values obtained by the two reducing procedures on unfermented plasma checked to within 5 per cent. The non-fermentable reducing substances in each case amounted to 10 per cent or less of the total reducing material.

For the fermentation technic 1 ml of a 20% Fleischmann's Baker's Yeast† suspension (previously washed 5 times with distilled water) is measured in a side arm of a 50 ml flask. In the bottom of the flask is placed 60 mg of pure glucose in 9 ml of water and 1 ml of plasma. The glucose is added in order to obtain sufficient CO_2 for counting purposes. The mixture is made acid to methyl red (pH 3) with a few drops of a saturated solution of citric acid. The flask contents are aerated with nitrogen for 5 minutes. The flask is then closed with a stopper having attached to it on a glass rod a center cup containing 1 ml of 5 N CO_2-free NaOH. After tipping the yeast suspension into the bottom of the flask the sample is incubated with shaking at 30°C for 3 hours. The carbon dioxide is obtained from the center cup by transferring the NaOH with three 1 ml water washes to a 15 ml centrifuge tube. The CO_2 is obtained as $BaCO_3$ by precipitation with 1 equivalent of 2 N NH_4Cl and 2 equivalents of 3 N $BaCl_2$ calculated for the NaOH. The $BaCO_3$ is washed three times with CO_2-free water and once with acetone, dried, and weighed.

Results and discussion. The method of calculating results is illustrated from the data on rabbit 37. The plasma glucose concentration by the Somogyi method was 121 mg % and the Miller-Van Slyke procedure gave values of 119 and 5 mg % before and after yeast fermentation. The value for the glucose concentration is then taken as 114 mg %. The radioactivity of the $BaCO_3$ obtained from fermentation of the mixture of 60 mg of pure glucose and 1 ml of plasma (1 ml plasma = 1.14 mg glucose) at infinite thickness was 544 cts/min/planchet. The calculated specific activity is 83.7. Dilution of the plasma glucose by the carrier glucose is $\frac{61.14}{1.14}$ or 53.6. Thus the specific activity of the plasma glucose is 53.6 x 83.7 or 4500. The specific activity of the injected glucose was measured on the $BaCO_3$ obtained by fermentation.

The data given in Table I for the insulin-treated animals show that the specific activities of the CO_2 obtained by fermentation of the plasma are nearly two times greater than that of the injected glucose. This is in marked contrast to the values obtained without insulin administration. It is obvious that insulin causes the appearance of a non-reducing fermentable substance which contains carbon 14. A comparison of the plasma glucose concentrations determined by reduction in comparison with the values calculated from the fermentable carbon 14 of the plasma show this in an even more striking manner.

Although further work will have to be carried out in order to identify the plasma substance, Van Slyke and Hawkins(6) have

3. Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 695.

4. Miller, B. F. and Van Slyke, D. D., *J. Biol. Chem.*, 1936, v114, 583.

5. Reinecke, R. M., *Am. J. Physiol.*, 1943, v140, 276.

† The yeast used in these studies was generously supplied by Standard Brands, Inc., San Diego, California.

6. Van Slyke, D. D., and Hawkins, J. A., *J. Biol. Chem.*, 1929, v83, 51.

TABLE I. Data Showing Appearance of a 'Fermentable Non-reducing Substance' in Plasma of Eviscerated and Normal Rabbits After 8 Hours of Constant Injection of C^{14} Labeled Glucose with Insulin Administration.*

Control						Insulin treated†					
Exp.	Days fast before evisceration	S.A. of CO_2 from plasma fermentation‡	S.A. of CO_2 from fermentation inj. glucose	Plasma glucose det. by reduction§ mg %	Plasma "glucose" calc. from fermentable C^{14} mg %	Exp.	Days fast before evisceration	S.A. of CO_2 from plasma fermentation‡	S.A. of CO_2 from fermentation inj. glucose	Plasma glucose det. by reduction§ mg %	Plasma "glucose" calc. from fermentable C^{14}‖ mg %
14	4	6300	8900	56	40	20	4	5760	3560	62	101
23	3	3540	4200	106	89	22	0	6770	3460	72	138
29	4	2540	2890	108	95	27	1	5015	2240	56	126
30	4	2560	2370	90	94	37	0	4500	3360	114	154
39	0	2500	2890	121	107	40¶	4	2930	2990	112	112
						36	1	2600	1670	150	226
						41	5	2810	1800	73	108
Avg		3488	4250	96	85			4340	2726	91	138

* In all of the experiments the rabbits were eviscerated with the kidneys left intact except experiments 36 and 41, where normal rabbits were used.

† 10 units of Iletin insulin were given I.V. at the start of each hour. We are indebted to Eli Lilly & Co., for generous supplies of insulin.

‡ Specific Activity (S.A.) is the cts./min./mg of carbon.

§ Difference between reducing values before and after fermentation.

‖ An example of this calculation using data for rabbit 37 is as follows: It is assumed that the Specific Activity of the CO_2 obtained from the plasma fermentation is that of the injected glucose and it could not be higher. Actually as can be seen from the values in the control rabbits it is about 20% lower. The S.A. of the fermentation CO_2 is then 3360 (same as the injected glucose) and the dilution of the plasma glucose by the carrier glucose is $x \times 83.7 = 3360$ or $x = 40$. (The S.A. constant of 83.7 is fixed if the counting is correct). The concentration of the plasma glucose under these conditions is 154 mg % ($\frac{60 + x = 40}{x}$) instead of the observed value of 114 mg %. Thus the concentration of the unknown compound appears to be present to the extent of the carbon in 40 mg % of glucose.

¶ We are unable to explain the negative results obtained with this animal. Sufficient plasma was not available to repeat the determinations.

pointed out that certain amino acids and α-keto acids have been shown to yield CO_2 under the influence of yeast carboxylase. They attributed the CO_2 from fermentable non-reducing compounds in urine to these sources.

We have made a preliminary survey of possible known substances which could arise from glucose. The following compounds were found not to be fermentable under the conditions reported here: lactic acid, succinic acid, 3-phosphoglyceric acid, glyceraldehyde, glycerol, α-ketoglutarate, dihydroxy acetone, glucose-1-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate. This at first seems very surprising since these phosphorylated compounds are intermediates in the fermentation of glucose and our yeast ferments glucose. This may be due to the fact that intact cells probably do not take up the phosphorylated compounds.

Summary. Data are presented showing that insulin induces the appearance of a 'fermentable non-reducing substance' in substantial quantity in the plasma of eviscerated and normal rabbits after 8 hours of constant injection of C^{14}-labeled glucose.

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