neutralized the virus; one partially neutralized and 2 failed to neutralize. Five of these 6 animals survived the intracerebral test.

Of the 26 normal controls employed in the intranasal tests, 25 succumbed. Of the 11 normal controls employed in the neutralization tests, all succumbed, as did the 13 normal controls used in the intracerebral tests.

Histologic study of the mucous membranes of the animals treated with pituitrin S and adrephine shows an extensive infiltration of the superficial and deep layers of the mucosa with eosinophiles. Some scattered eosinophiles are present in the submucosa, otherwise the mucous membranes appear normal. Mucous membranes obtained from normal untreated animals do not show eosinophilic infiltrations.

It appears from the results of these experiments, that not only do the majority of the animals develop appreciable protection against intranasal instillations of potent virus, but an appreciable number of these animals, particularly those included in the second group that had received prolonged daily treatments with pituitrin S and adrephine, as well as virus, developed an active immunity as indicated by the neutralization and intracerebral tests. These results help to bring out even a greater similarity between the experimental and the human disease, and perhaps serve to support the epidemiologic concept of the mechanism involved in the production of a widespread immunity to the disease in the normal population.[1] We do not attempt to explain the significance of the eosinophilic infiltration (or "barrier") in the mucous membranes of the treated animals.

9236 P

Effect of Pancreatic Tissue Extract on Cholesterol of Blood in Cardiovascular Arteriosclerosis.* †

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Pancreatic tissue extracts have been studied since 1908 by various investigators. Since 1929, insulin-free extracts have been investigated and independently prepared by Frey, Gley, Kraut, Kisthinios, Vaquez, and Wolffe. Wolffe and his associates reported that their

[1] Kramer, S. D., *J. A. M. A.*, 1932, **99**, 1048.

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pancreatic extract possessed vaso-dilator properties, which antagonized the pressor action of epinephrine and caused a transitory lowering of blood pressure in the animal. The commercially prepared pancreatic tissue extracts‡ contain traces of histamine, choline, salts, foreign proteins, and even slight traces of insulin. It has been demonstrated by the latter investigators, that the epinephrine neutralizing property of this substance is probably not due to these contaminants.[1]

Work has been carried on for some time on the effect of pancreatic tissue extract in cardiovascular arteriosclerosis manifested by angina pectoris or precordial pain and intermittent claudication in peripheral vascular diseases. During the course of this investigation an alteration in the blood cholesterol and phospholipids was observed. Before treatment in these cases, the cholesterol content of the blood was found to be rather high, the average being 275 mg. per 100 cc. Following the administration of pancreatic extract, it was noted that along with some clinical improvement, there was a tendency for the blood cholesterol to drop.

The Kirk, Page and Van Slyke gasometric microdetermination method was used for estimating the plasma lipids.[2] The percentage of error for blood cholesterol was found to be less than 2%.

In this investigation, the presence of choline in pancreatic tissue extract No. 568, was demonstrated by the Kraut test. Blood sugar concentration was not altered after the administration of pancreatic tissue extract. Blood sugar determinations were made by the Folin Wu method simultaneously with the lipids, fasting, one hour and 24 hours after the injection of pancreatic tissue extract.

A control experiment was carried out on 18 ward cases chosen at random. In these, fasting blood cholesterol concentration was determined according to the Bloor colorimetric method before, and one hour after, the injection of (a) 5 cc. aqua distillata in 3 cases; (b) 5 cc. physiological normal saline in 5 cases; and (c) 5 cc. pancreatic tissue extract in 10 cases. In (a) there was an average rise of 4.9 mg. cholesterol per 100 cc. blood, one hour after injection; in (b) an average rise of 20.6 mg.; and in (c) an average decrease of 38.8 mg.

Results are summarized in Table I. One hundred and eighteen

‡ Tissue Extract No. 568 was prepared according to the method of Wolffe (*cf.* Hyman and Parsonnet, *The Failing Heart of Middle Age*, 1932, p. 442), by Sharp and Dohme, for which I wish to thank them.

[1] Wolffe, J. B., *et al.*, reported before the 15th International Congress of Physiologists, Leningrad, August, 1935.

[2] Kirk, E., Page, I. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1934, **106**, 203.

TABLE I.

Effect of Tissue Extract on Total, Free and Esterified Cholesterol.
Columns marked (1)—represent Fasting concentrations.
" " (2)—one hour after injection of Pancreatic Tissue Extract.
" " (3)—24 hours " " " " " "
Esterified cholesterol may be calculated by subtracting Free Cholesterol from Total Cholesterol.

Subject Case No.	Date	cc. injected	Total Cholesterol			Free Cholesterol		
			1	2	3	1	2	3
1	10-20	10	270.0	195.1	214.8	65.8	90.9	74.8
2	10-6	10	196.4	230.0	254.2	91.2	68.8	71.1
3	8-20	5	306.5	213.7	—	115.5	110.6	—
	10-1	5	300.0	289.0	—	106.0	103.2	—
	10-29	5	279.5	260.0	270.0	145.0	138.5	121.5
	11-12	5	248.5	241.0	251.0	—	—	—
4	8-27	5	306.5	267.0	—	137.7	130.5	—
	10-1	5	243.0	210.0	—	163.5	120.9	—
	10-29	5	339.5	306.5	316.0	141.0	120.5	123.5
5	9-3	5	256.2	211.3	—	148.3	130.5	—
	10-1	5	335.0	318.0	—	136.0	159.0	—
6	10-22	5	373.5	341.0	328.5	163.0	148.5	146.5
	11-26	3	313.0	300.5	278.5	—	—	—

plasma cholesterol determinations were done on 23 patients after 46 injections. Forty-eight were done on fasting blood; 46 one hour after, and 24, 24 hours after injection of pancreatic tissue extract. In all but one case, a drop in the blood cholesterol was noted one hour after injection. Case 1 is a typical example. The exception (Case 2) showed a rise one hour after injection. There was a consistent fall in the blood cholesterol of 14 patients who had regularly received a 3 cc. injection 3 times a week for a period of time. In these, determinations were made at intervals of 3 to 5 weeks during the course of treatment. Case 3 is a typical example. Where the treatment was interrupted or dosage too small, *i. e.*, 1 or 2 cc. pancreatic tissue extract 3 times a week, there was an exacerbation of symptoms and an increase in plasma cholesterol. Such results were noted in 4 cases, demonstrated by case 4 (interrupted treatment) and case 5 (inadequate treatment). Of 24 determinations, on blood taken 24 hours after injection, 3 have shown a further fall below fasting and 21 tended to return almost to original level. Case 6 is a typical example of a continued fall 24 hours after injection.

The greatest drop in cholesterol in one hour was 97 mg. per 100 cc. blood or 31%. The mean decrease in cholesterol observed one hour after 5 cc. injection of tissue extract (37 injections) was 8.3%; the average deviation from the mean was 4.36%. The mean

decrease in cholesterol observed one hour after 10 cc. injection of tissue extract (9 injections) was 12.7%; the average deviation from the mean was 9.7%.

Summary. The results indicate that pancreatic tissue extract produces a lowering of the plasma cholesterol within one hour in cardiovascular arteriosclerosis. The cholesterol remains lowered, but with a tendency to return almost to former level in 24 hours. The effect is transitory, lasting about 24 hours, more or less. For sustained effect and progressive drop in plasma cholesterol, patients require doses at frequent intervals. When treatment is interrupted or dosage inadequate, the plasma cholesterol rises and there is an exacerbation of symptoms, precordial pain and intermittent claudication.

9237 P

Active Form of 2-4 Dinitrophenol in the Stimulation or Inhibition of Oxygen Consumption of Excised Rabbit Muscle.*

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Field, Martin and Field[1, 2, 3] have shown that the stimulation or inhibition of yeast respiration by 2-4 dinitrophenol (DNP) and several related compounds depends upon the concentration of the undissociated form. Since this finding appears to have important physiological and pharmacological implications, we have undertaken an investigation of the action of DNP on excised rabbit striated muscle (diaphragm) to see whether the undissociated form is the active agent in stimulation of oxygen consumption of a mammalian tissue as well as of yeast.

Rabbits were killed by a blow on the back of the neck. The diaphragm was rapidly excised, and strips of proper thickness (*c. f.* Warburg[4]) were placed in Ringer's solution containing 0.2% glucose and buffered at the desired pH with M/150 phosphate. Res-

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

1 Field, J., II, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

2 Field, J., II, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

3 Field, J., II, Martin, A. W., and Field, S. M., *J. Pharm. and Exp. Ther.*, 1935, **53**, 314.

4 Warburg, O., *Über den Stoffwechsel der Tumoren*, Berlin, 1926.