The only significant difference in response to the 10-2-88 mixture as compared with the response to the 12-0-88 mixture was the greater respiratory activity produced by the former. Therefore, the results indicate that no beneficial effects would be obtained by substituting carbon dioxide for oxygen when the original oxygen tension is insufficient for normal saturation of the hemoglobin of arterial blood. On the other hand, it is apparent that a considerable part of the oxygen in inspired air may be replaced with carbon dioxide without a reduction in alveolar oxygen tension.

Comparison of the effects of the last 3 mixtures listed in Table I indicates that increasing the carbon dioxide in the inspired air, when the oxygen tension of the inspired air is deficient but is kept constant, results in an increase in the alveolar oxygen tension on the basis of increased respiratory minute volume. This result is in accord with a number of observations reported in the literature of relief of hypoxia obtained by increasing the carbon dioxide tension of inspired air without a corresponding reduction of oxygen tension. However, the alveolar oxygen tension when breathing the 12-2-86 and 12-3-85 mixtures is not significantly higher in the average case than the alveolar oxygen tension that would be expected to result from breathing 14% and 15% oxygen respectively even if no respiratory stimulation occurred. This does not necessarily mean that the oxygen tension in tissues would be the same in each case.

Comment. In view of the above results, in the presence of a degree of hypoxia that will allow a "leveling off" of the blood pressure and heart rate and respiratory minute volume within a few minutes after the onset of the hypoxia, it appears unlikely that increasing the carbon dioxide tension of the inspired air will produce any beneficial results beyond that produced by addition of an equivalent oxygen tension. It is possible that a different result would be obtained in the presence of more severe hypoxia, but such a degree of hypoxia would be tolerable for only a brief time.

14140

The Vasodilator Properties of a Pancreatic Extract.*

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Since the demonstration by Frey and Kraut[1] of the presence of a depressor substance in urine and blood and the subsequent preparation of extracts of pancreas with similar effects upon blood pressure, many clinical trials have been made with pancreatic extracts. Most of the reports deal with the so-called circulatory hormone of the pancreas, kallikrein or "Padutin" (see Rigler[2] for review of literature). A deproteinated extract of pancreas,* free of insulin, choline esters, and histamine, has been tried with reported success in various clinical conditions. In many of the clinical studies, however, no convincing control observations were made, and the subjective response of the patient was often the accepted criterion.

* The pancreatic extract used is manufactured and supplied by Sharp & Dohme, Inc., under their trade name, "Depropanex" deproteinated pancreatic extract. It is stated to contain not more than 2.5% solids, including not more than 0.5% nitrogen, 0.9% sodium chloride, and 0.25% phenol as a preservative. This investigation was aided by a grant from Sharp & Dohme, through the courtesy of Dr. L. Earle Arnow.

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1 Frey, E. K., and Kraut, H., *Z. physiol. Chem.*, 1926, **157**, 32.

2 Rigler, R., *Heffter's Handb. exp. Pharmakol.*, 1938, **7**, 63.

Elliot and Nuzum,[3] comparing an insulin-free pancreatic extract with "Padutin," found that intravenous doses of either preparation in rabbits caused a brief fall in blood pressure and that repeated doses led to a more prolonged hypotension. In hypertensive patients intravenous administration of the extracts caused a fall in systolic pressure lasting as long as an hour; but no effect was observed on the blood pressure of normal human subjects and no effect was found following subcutaneous or intramuscular administration in either rabbits or human subjects. The ineffectiveness of intramuscular and oral administration to animals of large doses of an insulin-free pancreatic extract was also emphasized by Van Winkle.[4]

Since no studies had been made of the effect of pancreatic extract on blood flow, with the exception of the Langendorff perfusion experiments of Elliot and Nuzum, it seemed desirable to record the action of this material upon coronary blood flow in the dog heart-lung preparation and upon femoral arterial flow in cats in an attempt to establish whether or not there is any experimental basis for its clinical use.

I. *The effect of pancreatic extract upon coronary sinus outflow in the denervated and innervated heart-lung preparation. Methods.* Studies on the coronary circulation were carried out on the denervated heart-lung preparation of the dog. Dogs of both sexes, weighing 10 to 15 kg, were used under "Nembutal" or chloralose anesthesia. Defibrinated blood obtained from an etherized bleeder dog was used. Coronary sinus outflow was measured by means of a Morawitz cannula and a Condon flow recorder. Aortic pressure was recorded by means of a mercury manometer and was kept constant in all experiments.

Several experiments were performed with the innervated heart-lung preparation in which the head was perfused from a pump-oxygenator system; no vascular connection existed between the head and thorax.

Results. In 4 experiments single doses of pancreatic extract, resulting in concentrations‡ of from 1:1000 to 1:700 in the coronary blood, exerted no effect upon coronary flow. Great but transient increases in flow resulted when the extract was administered in concentrations of 1:200 to 1:100.

In one experiment an attempt was made to determine the minimum effective concentration. Pancreatic extract was injected into the inflow tubing from a constant rate infusion pump. As shown in Fig. 1, the initial sinus

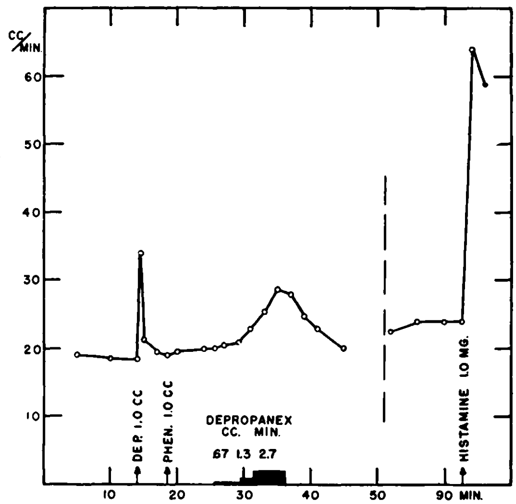

FIG. 1.
Coronary sinus outflow in the denervated dog heart-lung preparation. Injections directly into the venous inflow tubing. Arrow marked "Phen" signifies the injection of 0.25% phenol in saline.

flow was 19 cc per minute. An initial dose of 1.0 cc injected rapidly into the inflow tubing almost doubled the rate of blood flow, but for less than a minute. A control injection of 1.0 cc of 0.25% phenol (used as a preservative in the pancreatic extract) failed to produce any dilatation. Following this, pancreatic extract was infused at a rate of 0.66 cc per min for 4¼ minutes, a total dose of 2.84 cc. The total output of the heart was about 440 cc per minute; the concentration in the inflowing blood was therefore 1:660, assuming no cumulation in the blood. A negligible increase in coronary flow, less than 5%, occurred. The injection rate was then increased to 1.33 cc per minute, a concentra-

[3] Elliot, A. H., and Nuzum, F. R., *J. Pharmacol. Exp. Therap.*, 1931, **43**, 463.

[4] Van Winkle, W., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 220.

‡ Concentrations are stated in this report in terms of the volume of the pancreatic extract.

tion of 1:330, and the infusion continued for 2¼ minutes. As only a moderate increase in flow occurred, the injection rate was increased to 2.66 cc per minute, at a concentration of 1:165. The coronary flow increased gradually during the 4-minute infusion period to 29 cc per minute, an increase of 45% over the basal level. The flow returned to the control level in 9 minutes after the infusion was stopped.

Since coronary flow increased gradually during the infusion period, it is obvious that cumulation in the blood occurred; that is, the active substance was not destroyed by one passage through heart and lungs. However, in spite of a total dose of 18 cc (concentration of 2.7% in the entire blood volume) the flow rapidly returned to normal, indicating a rapid rate of inactivation or destruction. From this experiment it would appear that the minimal effective concentration in the denervated dog heart-lung preparation lies between 1:660 and 1:330, approximately 0.2%.

Later in this experiment 0.25% phenol in saline was infused for 4 minutes at 2.66 cc per minute without effect upon coronary flow.

In the experiments with the innervated heart-lung preparation comparable results were obtained. Pancreatic extract injected into the cardiac circulation in high concentration caused coronary dilatation, but no effects followed the injection of the extract into the cephalic blood supply, indicating that central or reflex factors are not concerned in the coronary dilator action of the substance.

II. *The effect of pancreatic extract upon peripheral blood flow. Methods.* Experiments on femoral arterial flow were performed on cats under ether, "Nembutal," or chloralose anesthesia; a stable control period was most regularly obtained with chloralose. The animals were heparinized (5 mg kg) and cannulas inserted into the left carotid artery and the right external jugular vein for the recording of mean arterial pressure and the intravenous administration of drugs. The flow-meter was inserted into the right femoral artery by means of centrally and peripherally directed cannulas. The flow-meter was a coil of glass tubing graduated at 2 and 4 cc and contained in a constant-temperature bath. The rate of passage of a bubble of air large enough to occupy the whole diameter of the tube was timed with a stop-watch between the graduations. An air-trap at the top of the instrument collected the bubbles. Readings could be taken with an intervening time of only 2 or 3 seconds and were repeated as often as possible when changes were expected. From 5 to 10 minutes were required for insertion of the bubble stromuhr, but, since no attempt was made to interrupt collateral circulation, the leg was not subjected to severe asphyxia, and the flow soon reached a stable level. Observations were also made of cephalic blood flow in the innervated heart-lung preparation.

Results. The effect of pancreatic extract upon the femoral arterial flow was studied in 4 experiments. Doses of from 0.005 to 0.02 cc of the extract made up to 0.1 cc in physiological saline were injected into the peripheral limb of the stromuhr directly above the cannula. Numerous control injections of saline and of 0.25% phenol in saline proved to be without significant action. No attempt was made to determine the limiting concentration of pancreatic extract; at the basal level of blood flow in the various experiments the doses used reached the vessels of the leg in concentrations of from 3 to 14% in the blood, assuming no dilution from collateral channels. Representative results are illustrated in Fig. 2. Great but transient increases in blood flow occurred in every case, increases ranging from 118% to 650% of the control level. Blood flow in each instance returned to normal within 3 minutes after the injection.

Comparison was made of the pancreatic extract activity with that of glyceryl trinitrate, histamine, and acetylcholine. Pancreatic extract, 0.01 cc, was found to be roughly equivalent to 0.02 to 0.04 mg of glyceryl trinitrate; to 0.001 mg of histamine hydrochloride; or to 0.05 μg of acetylcholine chloride. Atropine in doses sufficient to abolish the effect of 1 μg of acetylcholine did not reduce the response to pancreatic extract.

Pancreatic extract in doses of 0.005 to 0.01 cc intravenously was found to have little or no action upon blood pressure or femoral flow

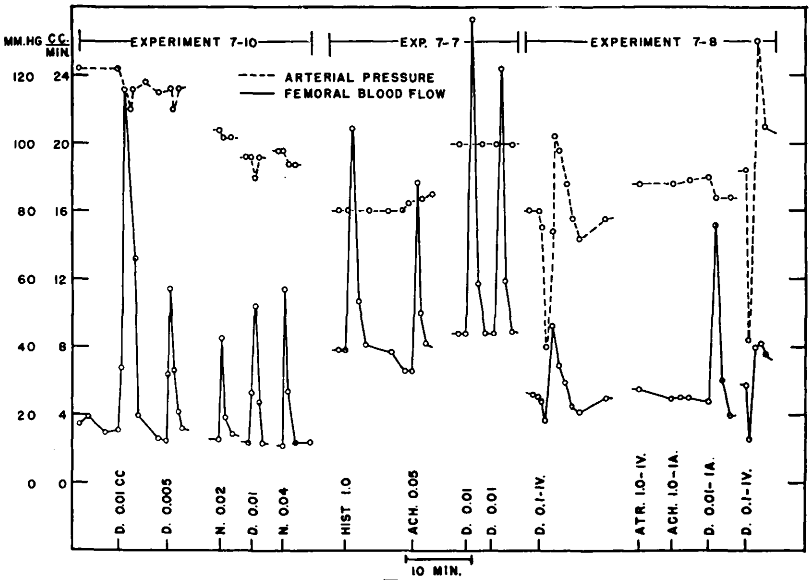

FIG. 2.

Arterial pressure and femoral arterial flow in anesthetized cats. D = pancreatic extract (dose in cc); N = glyceryl trinitrate (dose in mg); Hist. = histamine hydrochloride (dose in μg); Ach. = acetylcholine chloride (dose in μg; Atr. = atropine sulfate (dose in mg). All doses intra-arterial except those designated I.V. in the third experiment, which were intravenous.

in these cats, but in doses of 0.1 cc a profound fall in blood pressure always occurred. Mean arterial pressure fell from 80 to 42, from 80 to 40, from 92 to 42, and from 100 to 54 mm of Hg in 4 trials. The blood pressure decline lasted only one or 2 minutes and was followed by a rise of from 20 to 40 mm of Hg above the basal level, returning to normal within 4 or 5 minutes. Femoral flow after intravenous injection appeared to follow passively the changes in blood pressure. If vasodilatation did occur in the leg vessels, in no instance was it of sufficient degree to disturb significantly the positive correlation which existed between the blood pressure and femoral blood flow. If the blood pressure fall was caused by the vasodilating effect of the drug, it appears that the greater effect of the extract was exerted upon the splanchnic bed.

In the experiments upon the innervated heart-lung preparation the head was perfused through the brachiocephalic artery from a constant-output pump; changes in head perfusion pressure therefore reflected changes in vessel caliber in the head. Pancreatic extract, 1.0 cc, injected directly into the arterial stream, caused a transient but significant decrease in the perfusion pressure, indicating a dilatation of the cephalic arterial tree. In experiments in which the head perfusion pressure was kept constant and blood flow measured by means of a Weese stromuhr, 1.0 cc of pancreatic extract intra-arterially resulted in a transient increase in head blood flow. Concentrations were of the order of 1:400 to 1:200.

Conclusions. Although these experiments demonstrate that pancreatic extract exerts a vasodilator action upon the coronary, cephalic, and femoral arterial beds, the concentrations required are high (from 0.2 to 1%) and the duration of action very brief. The effects of large doses last, at most, 5 minutes. It is doubtful whether any increase in coronary flow would be observed with practicable doses in the intact animal; in the heart-lung preparation the aortic pressure is artificially maintained at a constant level, whereas the fall in blood pressure occurring in the intact animal would certainly decrease the magnitude of the coronary flow response. No evidence was found for any reflex change of coronary flow in the innervated heart-lung preparation. While no measurements of leg blood flow have been reported in human patients with peripheral vascular disease, it is doubtful that the recommended intramuscular doses could cause any change in flow in normal subjects.

Summary. 1. Pancreatic extract in concentrations of 0.2 to 1% causes a transient but significant increase in coronary sinus outflow in the denervated and innervated heart-lung preparation. 2. Femoral blood flow in anesthetized cats may be increased by 100 to 650% following doses of 0.005 to 0.02 cc of pancreatic extract injected intra-arterially. The effect lasts no more than 2 or 3 minutes. 3. Doses of 1 cc of pancreatic extract cause a transient dilatation of the arterial bed of the perfused dog head. 4. Doses of 0.01 cc have no regular effect upon arterial pressure when injected intravenously into anesthetized cats. Doses of 0.1 cc cause a 50% fall in arterial pressure lasting only one or 2 minutes, followed by a rise of 25 to 30% above the control level lasting for 3 or 4 minutes. Femoral blood flow appears to follow passively the arterial pressure; vasodilatation probably occurs chiefly in the visceral vascular beds.

The authors are indebted to Professor Otto Krayer for his aid and advice in the preparation of this report.

14141 P

Repair of Peripheral Nerves by Grafts of Frozen-Dried Nerve.*

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Orientation, growth rate and grouping of growing nerve fibers are determined by the biophysical and biochemical constitution of the interfaces along which they advance.[1] Their most favorable growth medium is degenerated peripheral nerve.[2] Accordingly, peripheral nerve fragments are the best means of bridging traumatic nerve gaps.[3] In mammals, autografts can be fully, homografts adequately successful;[4] heterografts are largely discredited.[4,5] Nerve grafting in man has met with the difficulties of (1) growth-obstructing fibrosis of the distal suture line, and (2) unavailability of grafts. The former may be obviated by resection of the barrier,[6] or possibly completely avoided if sutureless nerve splicing by arterial sleeves[7] should prove as successful in man as it has been in animals. The supply difficulty is more serious, since commonly no source other than the patient's own intact nerves is available. In order to avoid having to sacrifice a "minor" nerve for the repair of a more vital one, several authors have tried preserved or fixed nerves. Storage in petrolatum[8] leaves the grafts viable, but presumably not indefinitely. Alcohol-fixed grafts[8,9] lead to poor regeneration,[5] formalin-fixed grafts to complete failure.[9]

Basically, these past failures are attributable to the denaturation of the grafts. Therefore, one of us (P. W.) thought of trying a preserva-

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