virus is comparable to that seen in other studies (1,2,3).

The high level of prevaccination antibody to poliovirus reflects the high proportion of personnel entering military service with positive vaccination histories (14).

Summary. No interference was noted in the acquisition of neutralizing antibody to either attenuated poliovirus or adenovirus in a group of Marine recruits simultaneously vaccinated with attenuated adenovirus type 4 and trivalent poliovirus vaccines. This study would indicate that combined vaccinations with these agents is feasible in the military.

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Biosynthesis of Immunoreactive Insulin by the Duct-Tied Dog Pancreas* (32827)

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Several different systems have been described to study the biosynthesis of insulin through the incorporation of labeled precursors into the active hormonal fraction. These have included the use of the isolated islet tissue (Brockmann bodies) of the marine teleosts (1–3), rat or rabbit pancreas slices (4–6), human islet cell tumors (7,8), and the separated islet of the rat pancreas (8). Each of the methods has inherent limitations as mentioned previously (9). Some of the problems encountered have been circumvented by the use of the isolated, recirculated, perfused dog pancreas as a model system to study the incorporation of ^{14}C labeled amino acids into insulin (9).

Another approach to this problem is described in this paper which presents the results of incubating the duct-ligated "acinar free" dog pancreas with ^{14}C labeled amino acids. This preparation actively incorporates ^{14}C labeled amino acids into fractions that have insulin activity by both biological and immunological assays.

Methods. Pancreas preparation. Adult mongrel dogs of either sex were used. Each dog was anesthetized with pentobarbital sodium (30 mg/kg) and the pancreatic ducts were exposed, doubly ligated and cut under

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sterile conditions. After closing the wound, each animal was given 300,000 units of benzithine penicillin G (Bicillin). The animals were maintained for a period of 8 weeks on a diet of Purina dog chow supplemented with feedings of pancreatin.

Pancreas incubation. For each incubation three duct-tied dogs were anesthetized with pentobarbital sodium (30 mg/kg) following an overnight fast but with free access to water. A small piece of each pancreas was fixed in formalin and hemotoxylin–eosin slides were prepared for histological examination. The three "acinar free" pancreata were minced together with a razor blade and the total mince was divided into three equal aliquots of approximately 5 gm. Each aliquot was incubated in 15 ml of the incubation medium (100 ml of Krebs–Ringer bicarbonate buffer contained glucose, 2.3 mg/ml; cytidine, 3 mg; uridine, 3 mg; nicotinamide, 10 mg; 30% human serum albumin, 0.16 ml) at 37°C in a Dubnoff metabolic shaker for 4 hours. The gas phase was 95% O_2–5% CO_2. In each incubation one 5-gm aliquot of tissue served as the control, one aliquot was incubated with 3 mg of porcine somatotropin (STH) (obtained from General Biochemicals, 925 Laboratory Park, Chagrin Falls, Ohio 44022) and one 5-gm aliquot was incubated with 2,4-dinitrophenol at a final concentration of 0.25 m*M*. All tissues were incubated for 15 min in their respective media before the addition of 1 ml of the ^{14}C labeled amino acid mixture.[2] At the end of the incubation the tissue was separated from the incubation medium by centrifugation at 5°C. Insulin activity was extracted from both tissue and medium and concentrated on Sephadex columns as described previously (9).

Assays for insulin activity and radioactivity content. Each fraction of the tissue and medium extracts was assayed for immunoreactive insulin (IRI) by the method of Hales and Randle (10),[3] and for qualitative insulin-like-activity (ILA) by the hemidiaphragm and fat pad assays. The radioactivity of each fraction was determined on a Nuclear Chicago liquid scintillation counter. All methods used were as previously described (9).

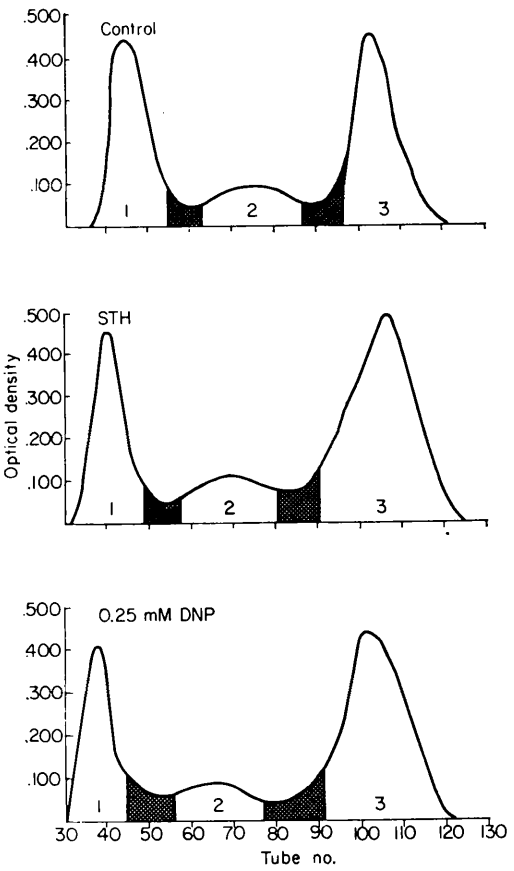

FIG. 1. Elution patterns of extracts from duct-tied dog pancreas. Sephadex G-50, 3 × 90-cm column, acetic acid.

Results. Elution patterns from Sephadex columns of pancreas extracts from a representative incubation (XV) are shown in Fig. 1. Each tube represents approximately 3.0 ml. The elution patterns of extracts from one incubation to the next were quite constant. To reduce overlapping of the fractions, the shaded area between each peak was discarded. In all cases three peaks were obtained and

[2] Uniformly labeled L-amino-^{14}C acids were obtained from Nuclear Chicago, Des Plains, Illinois. One ml of the mixture added contained 0.1 μC each of alanine, arginine, aspartic acid, DL cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, and tyrosine.

[3] We are grateful to Dr. Mary Root, Eli Lilly Research Laboratories, Indianapolis, Indiana for a generous supply of the guinea pig insulin antibody and for the crystalline porcine insulin Lot PJ 5589 used as the standard for this assay.

TABLE I. The IRI and ^{14}C Content of Extracts from Dog Duct Tied Pancreata.

	Peak 1	Peak 2	Peak 3
Immunoreactive insulin (units/mg of extract)			
Control			
XIV	0.010	0.058	*[a]
XV	0.009	5.500	.027
XVI	0.011	1.800	.019
STH			
XIV	0.013	0.025	*
XV	0.005	3.400	*
XVI	*	1.500	*
DNP			
XIV	*	0.012	*
XV	*	0.100	*
XVI	*	0.021	*
Radioactivity (cpm above background/mg of extract)			
Control			
XIV	1837	5028	4628
XV	1248	1720	8236
XVI	2193	2943	6384
STH			
XIV	2476	5597	6536
XV	1551	1746	8056
XVI	2749	5011	5521
DNP			
XIV	654	514	6984
XV	760	671	7303
XVI	530	259	6199

[a] * = undetectable levels of IRI.

designated 1, 2, and 3 from left to right. Crystalline porcine insulin eluted from the columns as a sharp peak in the midregion of peak 2, and we have assumed that peak 2 contained insulin. The inclusion of DNP or STH in the incubation media did not qualitatively alter the elution of the patterns of the pancreas extracts.

The results of the assays for IRI and the ^{14}C content of tissue fractions for three incubations are shown in Table I. The data reflect the great variability we have encountered in the use of this preparation. Variability is not unexpected since there were many uncontrollable factors in these animals such as age of the dogs and the precise nutritional status of the duct-ligated animals, etc. Another contribution to the variability are the problems of quantitative recovery of insulin from 5 gm of tissue. In spite of the variability from day to day, it was apparent that ^{14}C incorporation into the three fractions of control, STH, and DNP treated tissues could be compared for any given incubation. Therefore, the following generalities may be derived from the data presented.

In all cases peak 2 had the greatest amount of IRI compared to peaks 1 or 3. The activity in peak 2 ranged from 0.012 units/mg of extract in one DNP incubation to 5.5 units/mg in a control incubation. Small amounts of IRI were always found in peaks 1 and 3. Biological ILA assays were carried out on each fraction using the stimulation of glucose uptake of the hemidiaphragm and fat pad as the index of activity. Since these assays were used only for the qualitative rather than quantitative presence of ILA, the data are not shown; however, there was general agreement between the assays for IRI and ILA. The addition of STH to the incubation medium did not significantly effect the IRI or ILA content of the pancreas. However, the yields of IRI and ILA were invariably less than from control tissues when DNP was present.

In the control or STH treated tissue the radioactivity recovered in peak 2 was always equal to or greater than that recovered in peak 1. By comparison, DNP caused a marked reduction in ^{14}C incorporation into peak 1 and 2. However, the radioactivity recovered in peak 3 was comparable in control, STH and DNP treated tissues. The STH did not cause any consistent, significant change in the incorporation of ^{14}C into any of the fractions.

The pancreata used were considered to be free of acinar tissue. On gross observation of the duct-tied pancreas the gland was reduced in size from a normal 25–30-gm organ to a thin line of tissue lying along the duodenum and weighing about 5 gm. Histological examination of these glands revealed normal appearing islets of Langerhans and a plethora of fibrous tissue. Virtually no acinar tissue was present, but occasional acinar cells were found that had the histological appearance of being functional.

Discussion. The data presented indicate

that the surviving tissue of the duct-tied, acinar-degenerated, dog pancreas incorporates ^{14}C labeled amino acids into a fraction with insulin activity. Since the midportion of peak 2 eluted in the same volume as crystalline porcine insulin, and since peak 2 had the greatest amount of IRI and ILA, it is concluded that the incorporation of ^{14}C amino acids into this fraction represents the biosynthesis of insulin. DNP caused a marked reduction of incorporation of label into peak 2 as well as peak 1, and this is taken as evidence that the incorporation is an energy dependent process.

Peak 1 had marginal amounts of IRI and ILA that in no case approached the activity of peak 2. Peak 1 also contained significant amount of ^{14}C in the control and STH tissues which was much reduced in the presence of DNP. It has recently been reported on the basis of time labeling experiments with isolated rat pancreas islets and insulinoma slices that a "proinsulin" is formed which elutes from a Sephadex G-50 column in a peak preceding insulin (8). Whether the peak 1 reported here corresponds to "proinsulin" has not been determined since time labeling experiments have not yet been carried out.

Peak 3 always contained approximately the same amount of radioactivity regardless of the experimental conditions employed. It would be expected that this peak contained compounds of smaller molecular dimensions than insulin, and these counts probably represent smaller peptides and/or amino acids extracted along with the active fraction. The fact that DNP did not alter the amount of ^{14}C in this fraction indicates that the radioactivity found was independent of an energy source.

Over the years much evidence has accumulated to implicate pituitary STH in insulin synthesis and/or release by the pancreatic islets (11, 12). A more recent report has suggested that STH permits the pancreas to secrete increased amounts of insulin to a given stimulating glucose level (13). Martin *et al.* (14) have indicated glucose stimulates the incorporation of tritiated leucine into insulin as well as the release of insulin from isolated rat islet tissue. These workers showed that hypophysectomy reduced the responsiveness of the islets to glucose and that pretreatment with STH restored the synthesis and secretion of insulin to normal levels. In the data reported here, if each incubation is compared within itself, STH apparently reduced the IRI recovered in peak 2 without a corresponding reduction of the ^{14}C activity in this fraction. The uncertainty of the quantitative recovery of insulin from 5-gm aliquots of pancreas coupled with the fact that our best fractions had an activity of 5.5 units/mg while it is generally accepted that pure insulin has an activity of 20–25 units/mg renders interpretation of this apparent effect without value.

The duct-tied "acinar free" pancreas offers a biological system for the study of the biosynthesis of insulin which potentially has some advantages over other preparations used. Since only minimal amounts of histologically, viable-appearing acinar tissue were found, the preparation may be considered to be nearly free of contaminating pancreatic enzymes which may cloud results of rat or rabbit pancreas slice incubations which have been used in the study of insulin biosynthesis (4, 5, 6). This preparation provides greater amounts of "pure" islet tissue than may be obtained by the use of the geographically limited, marine teleost Brockmann bodies (1–3) or dissected islet tissue of the rat (8). As pointed out by Steiner *et al.* (8), the use of surviving human islet tumors is so severely restricted by frequency of the tumors and the coexistence of facilities to study insulin biosynthesis in surviving slices as to preclude more than an occasional observation. Any observation with the tumor tissue is, perforce, limited to a single tissue donor at a time. In addition, β-cell neoplasms may or may not synthesize insulin exactly as do normal β cells.

It is concluded that the duct-tied, acinar-degenerated pancreas of the dog may be used to study the incorporation of labeled precursors into fraction(s) with insulin activity.

Summary. "Acinar free" pancreata have been prepared by ligating the pancreatic ducts of dogs under sterile conditions and allowing the acinar portion of the pancreas to degenerate over the succeeding 8 weeks. Three

pancreata were minced together for each incubation, and the pooled mince was divided into three equal aliquots. One aliquot served as the control, one was incubated with STH, and one with DNP. The ^{14}C labeled amino acids were added to the incubation mixture and the tissues were incubated for 4 hours. Insulin activity was extracted from each tissue and medium and concentrated on Sephadex G-50 columns. Assay for ^{14}C, IRI, and ILA indicated that the greatest amount of IRI and ILA was found in the fraction from the column that eluted in the same position as crystalline insulin. This fraction in the control and STH tissues had an appreciable amount of ^{14}C. It is concluded that the duct-tied pancreas preparation is a useful model to study insulin biosynthesis. The presence of STH did not alter the ^{14}C incorporation or the recovery of IRI or ILA in this preparation.

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The Effects of 6-Thioguanine and Endotoxin on Experimental St. Louis and Japanese B Encephalitis Virus Infections in Chiroptera* (32828)

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Variations in the susceptibility of different species of insectivorous bats to experimental infection with certain strains of arthropod-borne viruses and the failure of susceptible species to show overt signs of encephalitis even when high levels of virus were demonstrable in brains and other tissues have been reported (1). These observations prompted additional studies designed to determine factors which might alter the response of resistant species or precipitate symptoms and death in susceptible species. Experiments with gravid bats demonstrated that the physiological stress of pregnancy did not alter the course of infection with strains of Japanese B encephalitis (JBE) or St. Louis encephalitis (SLE) viruses (2). In a correlated study it was shown that administration of cortisone did not increase the susceptibility of *Tadarida b. mexicana* to a strain of SLE virus of low infectivity for this bat species (Sulkin S. E., Sims, R. A., and Allen, R., unpublished observation). The increased body temperature and metabolic rate resulting from the maintenance of bats at 37°C caused a more rapid

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