secretion remains to be investigated.

The present findings are not incompatible with the effects observed after repeated administration of exogenous insulin *in vivo*. Several investigators have reported a decline in the insulin content of the pancreas(8,9). In such animals, hypoglycemia has been reported, and this could explain the reduced insulin content of the pancreas, for Parry and Taylor(10) have recently shown that insulin synthesis in isolated pancreatic tissue is related directly to glucose concentration in the incubation medium.

Summary. Isolated Islets of Langerhans from the rat's pancreas were incubated in media containing varying combinations of glucose, rat insulin, and guinea pig anti-insulin serum (GPAIS). Neither insulin nor GPAIS modified the rate at which insulin was secreted in response to glucose. The concept that insulin in the fluids surrounding Islet tissue can inhibit secretion of insulin by the β-cells is discussed in the light of these results.

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1. Malaisse, W., Malaisse-Lagae, F., Wright, P. H., Endocrinology, 1967, v80, 99.
2. Lacy, P. E., Kostianovsky, M., Diabetes, 1967, v16, 35.
3. Wright, P. H., Malaisse, W., Diabetologia, 1966, v2, 178.
4. Hausberger, F. X., Ramsay, A. J., Anat. Rec., 1952, v112, 341.
5. Logothetopoulos, J., Kaneko, M., Wrenshall, G. A., Best, C. H., in Metabolism of Pancreatic Islets, Brolin, S. E., Hellman, B., Knutson, H., ed., Pergamon Press Ltd., London, 1964, 333..
6. Frerichs, H., Reich, U., Creutzfeldt, W., Klin. Wschr., 1964, v43, 136.
7. Logothetopoulos, J., Davidson, J. K., Haist, R. E., Best, C. H., Diabetes, 1965, v14, 493.
8. Batts, A., Ann. N. Y. Acad. Sci., 1959, v82, 302.
9. Best, C. H., Haist, R. E., J. Physiol. (London), 1941, v100, 142.
10. Parry, D. G., Taylor, K. W., Biochem. J., 1966, v100, 2C.

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New Metabolizable Immunologic Adjuvant* for Human Use. 6. Disposition of Radioactivity after Administration of Labelled Vaccine To the Rat. (31774)

CURT C. PORTER AND DAVID C. TITUS (Introduced by M. R. Hilleman)
Merck Institute for Therapeutic Research, West Point, Pa.

Previously, it was shown(1-3) that emulsions prepared with Adjuvant 65 were almost as effective immunologically as preparations which contained mineral oil, but were considerably less irritating. Since all of the components of the adjuvant are forms of normally occurring dietary substances, they should be either metabolized or excreted by the animal organism. Histologically, it was shown that the initial deposits in the muscle became diffusely encapsulated and gradually disappeared. However, there was no information on the manner in which the components were disposed of after injection as the unique adjuvant-vaccine formulation. Consequently, 3 different preparations, each containing one of the adjuvant substituents labelled with carbon-14, were administered to rats. From radio counts of the injection sites, carcasses and excreta, it is concluded that the adjuvant is absorbed, and its components are metabolized or excreted.

Materials. Aluminum monostearate carboxy-C^{14} was supplied by Dr. C. Rosenblum, Merck Sharp & Dohme Research Laboratories, Rahway, N. J. It had a melting point of 190-194°, and the ash content,

* Adjuvant 65.

judging from dummy runs, was within the range 14.5-16%, which is typical of commercial products. Its specific activity was about 2.9 μc/mg.

Arlacel A (mannide monooleate), prepared from mannitol-1-C^{14}, was supplied by Atlas Chemical Industries, Wilmington, Del. Its specific activity was 1.68 μc/mg.

Glyceryl tri(oleate-1-C^{14}) was obtained from Nuclear-Chicago, Des Plains, Ill., and was estimated by chromatographic methods to be more than 96% pure. Its specific activity was 15 μc/mg.

Preparation of Adjuvant 65 and vaccine. The adjuvant is composed of 85.0% peanut oil, 10.6% Arlacel A and 4.4% aluminum monostearate. In a typical run, 1.0652 g peanut oil, 0.1332 g Arlacel A and 0.0554 g aluminum monostearate were mixed in a test tube and the temperature raised at the rate of about 4° per minute to 120°. The mixture was constantly stirred with a glass rod during the heating process. The homogeneous material was transferred to a calibrated emulsifier syringe, an equal volume of killed aqueous polyvalent influenza virus vaccine was added and the mixture thoroughly emulsified. The thick cream was extruded into 0.25 ml syringes, about 0.15 ml being placed in each syringe, and stored at 4° until used.

In preparing the labelled peanut oil vaccine, peanut oil was added to the benzene solution of about 33 mg C^{14}-triolein, as it was received from the manufacturer, and the benzene evaporated in a stream of nitrogen at 40°.† The other ingredients were then added, and the vaccine prepared as above. This preparation of Adjuvant 65 contained 2.6% triolein, 82.4% peanut oil, 10.6% and 4.4% Arlacel A and aluminum monostearate, respectively.

Methods. Male Sprague-Dawley rats, weighing 125 to 150 g each were given single intramuscular injections of 0.01 ml of the adjuvant vaccines. Injections were into the dorsal musculature of the right leg and the injection needle was left in place for 20 seconds to allow the viscous material to flow. Reproducibility of dose measurement was satisfactory, since the standard deviation of radioactive counts of replicate samples around the mean was ± 4.3% of the mean.

Rats which received the injections were placed in all-glass metabolism cages, for 24-hour periods, at various times following vaccine administration. Expired CO_2 was trapped in 1 N KOH, and aliquots of the alkaline solution counted by liquid scintillation. Urines and suspensions of feces, the latter decolorized by heating with alkaline H_2O_2 (4), were also counted. Animals were killed at various times, and the right legs were treated separately from the remainder of the carcasses. The tissue was dissolved by heating with 9 volumes of 1 N KOH, and aliquots of the digests were treated with H_2O_2 before counting. The carcass digests were composed of 2 phases, aqueous and fatty, so that accurate aliquoting was impossible. Consequently, these digests were extracted with petroleum ether, aliquots of the remaining aqueous phase and of the extract being decolorized and counted separately.

Counts in the right legs of animals taken immediately after injection of labelled vaccine were used as the baseline in all calculations; and values reported represent means from 4 animals.

Results. Labelled peanut oil. Radioactivity in the injected leg disappeared slowly (Table I), some 50% remaining after 37 days, and 27% after 121 days. The carcass contained from 1 to 6% of the dose at any one time, the amount being greatest 37 days after vaccine administration (Table II).

TABLE I. Radioactivity at Injection Site After Administration of Labelled Adjuvant 65 Vaccine.

Time after dose, days	% of dose in injected legs		
	Peanut oil	Arlacel A	Aluminum stearate
2	78.0	55.3	35.7
5	78.7	47.7	22.9
13	81.2	26.4	39.3
30			22.9
37	50.2	10.3	
72	44.8	14.5	15.4
121	27.4		
Se	3.6	1.8	3.1

† Evaporation of the benzene from the labelled material and subsequent addition of peanut oil and the other ingredients failed to yield a uniform vaccine probably because of poor mixing.

TABLE II. Radioactivity in Carcass (Excluding Injection Site) After Administration of Adjuvant 65 Vaccine.

Time after dose, days	% of dose in carcass (excluding injected leg)		
	Peanut oil	Arlacel A	Aluminum stearate
2	1.04	2.30	9.80
5	1.21	.93	17.79
12	2.08	.71	22.83
30			19.83
37	5.70	.39	
71	2.89	.03	7.85
Se	.35	.01	1.00

Relatively small quantities of radioactivity were excreted in the urine (Table III), but labelled CO_2 was constantly expired (Table IV). It is estimated that at the end of 14 days when 80% of the injected radioactivity was still at the injection site, about 3% was elsewhere in the carcass or had been excreted in the urine, while the remainder had been expired as $C^{14}O_2$.

Labelled Arlacel A. Radioactivity disappeared from the injected leg rapidly at first, and then more slowly (Table I); 13 days after vaccine administration only 26% of the label remained at the injection site, and 72 days after injection 10 to 15% remained. The rest of the body rapidly picked up a small amount of radioactivity (Table II), and a small amount of the absorbed material appeared in the expired CO_2 (Table IV). The greatest amount of radioactivity was found in the urine (Table III). In 14 days, when some 75% of the radioactivity had disappeared from the injection site, one to 2%, at the most, appeared in the carcass and expired CO_2, the bulk being found in the urine.

TABLE III. Urinary Excretion of Radioactivity After Administration of Adjuvant 65 Vaccine.

Time after dose, days	% of dose excreted in urine/day*		
	Peanut oil	Arlacel A	Aluminum stearate
0- 1	.03	19.19	.16
1- 2	.05	4.63	.14
7- 8	.13	1.84	.27
13-14	.06	.68	.12
22-23	.05		.08
29-30	.05		.08
36-37	.03	.19	
48-49	.01	.10	.03
70-71	.02	.10	.00
Se	.005	.05	.01

* Amounts of radioactivity found in feces were small and variable, frequently being due to urine contamination. Since the total quantities involved were insignificant, they are not considered in evaluation of the data.

Labelled aluminum stearate. This material, like Arlacel A, left the injection site rapidly at first, 64% being lost in the first 24 hours, and then more slowly so that 72 days after injection about 15% remained unabsorbed (Table I). Relatively large amounts of C^{14} were detected in the carcasses (Table II), ranging from 8 to 23% of the injected radioactivity, depending upon the time. A small amount of radioactivity was excreted in the urine (Table III), but essentially all of that not found at the injection site or the remainder of the carcass appeared as expired $C^{14}O_2$ (Table IV). Thus, in the first 14 days, possibly 2% of the injected radioactivity was excreted in the urine, 50% as CO_2, and 23% was found in the carcass. At this time about 40% of the administered material remained at the injection site.

TABLE IV. Radioactivity in Expired CO_2 After Administration of Labelled Adjuvant 65 Vaccine.

Time after dose, days	% of dose expired in CO_2/day		
	Peanut oil	Arlacel A	Aluminum stearate
0- 1	.56	.28	6.19
1- 2	1.34	.13	4.20
7- 8	2.94	.10	4.28
14-15	1.55	.11	1.79
22-23	1.03		2.28
29-30	1.30		1.78
36-37	.78	.06	
48-49	.43	.07	.70
70-71	.27	.09	.24
Se	.13	.001	.006

Discussion. It is clear that after intramuscular injection of Adjuvant 65 vaccine, the Arlacel A and aluminum stearate components are rapidly leached out of the emulsion. The Arlacel A, labelled in the mannose portion of the molecule, is either excreted in the urine as such or after metabolism. Since little radioactive CO_2 was expired, it is probable that if metabolism of the Arlacel A did occur, it was no more extensive than splitting to the carbohydrate and fatty acid portions, the former being excreted; the latter, being a naturally occurring acid, entered normal metabolic

pools. The stearic acid portion of the aluminum stearate was extensively metabolized as judged by the appearance of $C^{14}O_2$ in expired air. Although the fate of the aluminum is not known, it seems reasonable that it also would be leached out of the vaccine emulsion. Since the metal is a normal constituent of the tissues and is excreted in the urine [see (5)], presumably the injected material would be disposed of *via* this route.

Although only the triolein portion of the peanut oil was labelled, since the fatty acids of the oil contain about 56% oleic acid, the fate of the labelled substituent should be representative of the oil as a whole. The persistence of the radioactivity administered as peanut oil C^{14} vaccine at the injection site is consonant with the microscopic observation of encapsulated material long after administration(2). Nonetheless, radioactivity gradually left the injection site, and the triolein was metabolized, as shown by the expiration of $C^{14}O_2$.

Therefore, the evidence is good that all of the fatty components of the Adjuvant 65 vaccine were absorbed from the site of intramuscular injection, metabolized, and the metabolites either excreted or taken into normal body pools.

Summary. Three batches of Adjuvant 65 were prepared, each labelled with C^{14} in one of the components, and emulsified with aqueous suspensions of killed influenza virus. The resulting vaccines were administered intramuscularly to rats, and the disposition of the tracer was followed for 120 days. The C^{14} from aluminum monostearate-1-C^{14} and from Arlacel A (mannide-1-C^{14}-monooleate) disappeared from the injection site more rapidly than C^{14} from peanut oil (oil containing a small excess of glyceryltrioleate added as carboxyl C^{14}-labelled triglyceride). Expiration of $C^{14}O_2$ by the rats which were injected with labelled aluminum stearate or labelled peanut oil vaccines demonstrates that these fatty materials reached the metabolic pools and were metabolized normally. Radioactivity injected with the vaccine as mannide-labelled Arlacel A did not accumulate in the tissues, most of it being excreted *via* the urine. It is concluded that the fatty components of the Adjuvant 65 vaccines were absorbed after intramuscular administration and were disposed of *via* normal metabolic routes.

1. Woodhour, A. F., Metzgar, D. P., Stim, T. B., Tytell, A. A., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1964, v116, 516.

2. Peck, H. M., Woodhour, A. F., Metzgar, D. P., McKinney, S. E., Hilleman, M. R., ibid., 1964, v116, 523.

3. Stokes, J., Weibel, R. E., Drake, M. E., Woodhour, A. F., Hilleman, M. R., New. Engl. J. Med., 1964, v271, 479.

4. Herberg, R. J., Anal. Chem., 1960, v32, 42.

5. Campbell, I. R., Cass, J. S., Cholak, J., Kehoe, R. A., A. M. A. Arch. Ind. Health, 1957, v15, 359.

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Calcium and Phosphate Dependency of Amino Acid Transport in Rat Kidney-Cortical Slices *in vitro*.* (31775)

DAVID M. BROWN† AND ALFRED F. MICHAEL‡ (Introduced by R. A. Good)
Department of Pediatrics, University of Minnesota Hospitals, Minneapolis

The association of increased urinary amino acid excretion in disorders of Ca^{2+} and phosphorus metabolism including vitamin D deficient rickets(1) and the Fancone syndrome(2) and the competition between amino acids and inorganic phosphate (P_i) for renal tubular absorption(3,4) have prompted an *in vitro* study of the dependency of amino

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† USPHS Trainee. Present address: 207 Ferson Loop, San Antonio, Texas 78236.

‡ Established Investigator of Am. Heart Assn.