lation between body weight and liver weight may change.

In clinical malnutrition the fall in plasma copper may be due to diminution of a_2 globulin and a fall on ceruloplasmin has been reported(3). If ceruloplasmin is formed in the liver as are many other a_2 globulin complexes then at least in the early stages of experimental malnutrition there is no lack of sufficient copper relative to liver and body weight to supply the copper for this synthesis.

Summary. Malnourished rats have a lower total liver copper than normal rats. However there is no significant change in copper content per 100 g liver or per g body weight.

1. Stransky, E., Dauis-Lawas, D. T., Lawas, I. L., *Ann. paediat.* 1952, v179, 1.
2. Lahey, M. E., Behar, M., Viteri, F., Scrimshaw, N. S., *Pediatrics,* 1958, v22, 72.
3. Reiff, B., Schnieden, H., *Blood,* 1959, v14, 967.
4. Adams, C. W. M., Fernand, V. S. V., Schnieden, H., *Brit. J. Exp. Path.,* 1958, v39, 30.
5. Bruce, H. M., Parkes, A. S., *J. Anim. Tech. Assn.,* 1956, v7, 54.
6. Adams, C. W. M., Fernand, V. S. V., Schnieden, H., *J. Endocrin.,* 1960, v21, 137.
7. Deegan, T., Maegraith, B. G., *Ann. Trop. Med. Parasit.,* 1958, v52, 232.
8. Williams, T. R., Morgan, R. R. T., *J. Chem. and Indust.,* 1954, v16, 461.

Received December 15, 1961. P.S.E.B.M., 1962, v110.

Determination of Phosphoric Acid Esters in Muscle of Mice Inoculated with Influenza Virus. (27513)

LUCIA B. DE GUERRERO AND ARMANDO S. PARODI (Introduced by G. Middlebrook)
Catedra de Microbiologia y Parasitologia, Facultad de Medicina, Universidad de Buenos Aires, Argentina

Results have been presented(1) of the study of concentrations of inorganic and organic phosphorus present in the allantoic fluid of chick embryos infected with influenza virus, Type A. It was observed that concentrations of phosphocreatine and total phosphorus were significantly decreased. The present work is concerned with the results of measuring inorganic phosphorus, phosphocreatine, ATP and total phosphorus in muscle of mice infected with an asiatic strain of influenza virus, A_3/Formosa/57, not adapted to mice.

Materials and methods. Animals. One group of adult white mice was inoculated i.p. with 0.2 ml of normal allantoic fluid; a second group, with allantoic fluid infected with the virus (hemagglutinating titer, 1:1280) and a third group, with virus plus specific antiserum. This last mixture was also inoculated into chick embryos and infectivity was shown to be completely neutralized. The animals were sacrificed at 24, 48 and 72 hours after inoculation; they were anesthetized with pentothal and immersed in a bath of ether and dry ice. A piece of frozen muscle from the leg was excised and weighed. The muscle was deproteinized with 5% cold trichloroacetic acid, and the protein-free filtrate was used in the following determinations of phosphorus.

Phosphorus analyses. a) *Inorganic phosphorus*: was carried out according to the Lowry-Lopez method(2). b) *Phosphocreatine phosphorus*: the protein-free filtrate was left for 2 hr at room temperature and inorganic phosphorus was measured in an aliquot, according to the method of Fiske-Subbarow(3). c) *ATP phosphorus*: hydrolysis of the protein-free filtrate was carried out 10 minutes at 100°C with 1N HCl. P was determined as in b). d) *Total phosphorus*: acid hydrolysis was continued for 3 hr, then phosphorus determined as in b).

Muscle glycogen determination. A piece of muscle was treated with hot 30% KOH; 1.1 volume of absolute alcohol was added. The mixture was centrifuged and the supernate discarded. The precipitated glycogen was hydrolyzed with acid, and glucose determined according to Valverde(4).

Specific antiserum. An adult rabbit was

TABLE I. Phosphorus Determination (mg of P per 100 g of Muscle).

Inoculum	Hr after inoc.	Inorganic phosphorus	t	P from phospho-creatine	t	P from ATP	t	Total phosphorus	t
Normal animals		31.6 ± 5.6		31.0 ± 4.9		33.3 ± 5.9		105.7 ± 9.8	
#F	24	31.6 ± 4.9	0	26.8 ± 3.6	2.4	22.3 ± 3.1	7.1	89.2 ± 6.0	3.7
	48	28.2 ± 5.7	1.71	24.6 ± 3.6	3.6	22.8 ± 2.8	17.5	88.3 ± 9.6	4.5
	72	29.4 ± 4.2	1.20	20.9 ± 4.1	16.87	24.4 ± 3.6	4.96	83.8 ± 7.8	5.5
Normal allant. fluid	24	30.9 ± 8.9	.29	30.8 ± 4.3	.24	29.6 ± 3.7	1.97	101.2 ± 8.8	1.0
	48	30.8 ± 4.0	.49	37.6 ± 6.4	2.50	31.6 ± 4.0	.75	111.1 ± 8.5	1.3
	72	31.7 ± 6.3	.03	34.7 ± 5.1	2.00	29.8 ± 3.7	1.9	104.4 ± 7.0	.3
#F + anti-serum	48	35.2 ± 4.1	1.68	20.3 ± 6.1	4.58	24.7 ± 7.9	3.64	95.8 ± 7.2	1.40

inoculated i.m. with 0.5 ml of infected allantoic fluid plus 1 ml of Freund's adjuvant. After 15 days the animal was bled and the serum was tested for ability to neutralize infectivity on chick embryos.

Infectivity in muscle of inoculated mice. Pieces of muscle were taken from mice inoculated i.p. with influenza virus. They were ground in a mortar and extracted with broth. After centrifugation, the broth was diluted and inoculated into 11-day-old chick embryos.

Results. Averages and standard deviations of each series (15 or more animals per group) of determinations are presented in Table I. A strong decrease in phosphorus from PC, ATP and total ester occurred at 24, 48 and 72 hr after inoculation of infected allantoic fluid as shown in Table I. This was not observed in the control animals. The data for muscle glycogen are shown in Table II. No significant differences were found between infected and the normal animals. The muscle extracts inoculated into chick embryos had no infectivity. At time of death there was no evidence of infection or toxicity in the mice of the second or third groups.

TABLE II. Glycogen Determination (mg of Glycogen per 100 g of Muscle).

Inoculum	Hr	Glycogen min max
#F	48	240 - 480
	72	220 - 450
	96	170 - 350
	120	220 - 350
	144	230 - 470
N.A.F.	48	290 - 400
	72	220 - 490
	96	170 - 400
	120	220 - 350
	144	280 - 450
Controls		220 - 485

Discussion. Several authors have found alterations in the phosphorus metabolism of infected chick embryos. Pappalardo(5) found an increase in creatine and creatinine levels in infected allantoic fluids. Parodi(1) observed a strong decrease in phosphocreatine, inorganic phosphorus and total acid soluble phosphorus in infected chick embryos.

In the present work a decrease was found in phosphocreatine, ATP, and total acid soluble phosphorus in mice inoculated i.p. with influenza virus. Control animals inoculated with normal allantoic fluid did not show any alteration from normal values. Muscle from infected animals proved not to be infective. Moreover no variation was found in muscle glycogen in infected animals. As already known phosphocreatine is the source for ATP resynthesis. Thus a decrease in both values could be the result of inhibition of phosphocreatine synthesis. A similar effect has been found in iodoacetate "poisoned" muscle. In this case glyceraldehyde-3-phospho-dehydrogenase is inhibited, as is the resynthesis of ATP from ADP.

Since muscle manifested no infectivity it is possible to attribute this action to virus toxicity. Until recently toxicity was considered to be due to the entire virus particle. But Kato and Okada(6) have found that sonic disintegration of influenza virus results in an increase in toxic activity of filtrates, in spite of the loss of infectivity. They suggested that toxicity is due to a protein or a lipoprotein strongly bound to the hemagglutinin, but

a small part of it remains with the *s* antigen.

Henle and other investigators reported that toxic activity of the total virus particle is neutralized by the specific antiserum. In our experiments, however, only the decrease in total phosphorus was neutralized by the specific antiserum.

A similar variation in phosphocreatine level was found by Pinchot and Bloom(7) after inoculating diphtheria toxin into rats subcutaneously, but this action was neutralized by the antitoxin.

Perhaps the toxic activity observed in our work can be attributed to one of the virus components against which antiserum has no action. On the other hand, the possibility of an interferon action must be considered. It is already known that normal cells inoculated with interferon increase their oxygen uptake and their lactic acid production, but glycolysis is related to ATP production. It seems fair to suggest that this is the way interferon acts against the virus, lowering the ATP level, so that virus synthesis cannot be accomplished(8). In any event interferon produced by the inoculated animals could be the agent responsible for the ATP and phosphocreatine decrease.

Conclusions. 1. Analysis of inorganic phosphorus, phosphocreatine, ATP and total acid soluble phosphorus was carried out in white mice previously inoculated with influenza virus and also in control animals. 2. A significant decrease was observed in muscle phosphocreatine, ATP and total phosphorus in the infected animals.

1. Parodi, A. S., *Arch. Biochem.,* 1949, v22, 324.
2. Lowry, O. H., Lopez, J. A., *J. Biol. Chem.,* 1946, v162, 421.
3. Fiske, C. H., Subbarow, Y., *ibid.,* 1925, v66, 375.
4. Valverde, E., *An. As. Quim. Arg.,* 1959, v47, 135.
5. Pappalardo, M. H., *Rev. Inst. Bact. Malbrán,* 1949, v14, 16.
6. Kato, N., Okada, A., *Brit. J. Exp. Path.,* 1961, v42, 253.
7. Pinchot, G. B., Bloom, W. L., *J. Biol. Chem.,* 1950, v184, 9.
8. Isaacs, A., *Virology,* 1960, v10, 144.

Received April 23, 1962. P.S.E.B.M., 1962, v110.

Effect of Adrenalectomy and Corticoid Replacement on Lactational Performance in Rats.*† (27514)

R. R. ANDERSON‡ AND C. W. TURNER
College of Agriculture, University of Missouri, Columbia

Importance of adrenocortical hormones in maintenance of lactation has been(1-4) unieequivocally substantiated in adrenalectomized rats. Levels of corticoids required for complete maintenance of lactation have been found to be 0.85 mg cortisone acetate and 0.36 mg desoxycorticosterone acetate (DOCA) per day in rats adrenalectomized on day 4 of lactation as judged by absorption of pellet implants(5). On daily injection basis, 1.1 mg cortisone and 0.43 mg DOCA per day maintained normal lactation(6).

Maintenance of normal lactation in adrenalectomized rats was accomplished with 3 mg DOCA per day while 0.1 or 1 mg were not sufficient(3). Maintenance of lactation in rats at 80% of normal was obtained with 0.46 mg cortisone per day(2). However, other investigations found that 0.5 mg 11-dehydrocorticosterone or 0.47 mg cortisone did not maintain lactation(4).

9α-Chlorocortisol at 100 μg per day maintained almost complete lactation; aldosterone at 50 μg per day maintained lactation at a lower level(7). 9α-fluorocortisol acetate at 200 μg per day maintained normal lactation

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2395. Approved by the Director.
† Aided in part by research grants from P.H.S. and Am. Cancer Soc.
‡ Author was predoctoral fellow of N.I.H. during period of this investigation.