treated controls. Similarly, hypophysectomy before 1:30 p.m. inhibited ovulation while hypophysectomy between 5:00 and 5:30 p.m. did not. These data are interpreted to mean that ovulating hormone is released from the pituitary gland between 1:30 and 5:30 p.m. on the 24th day of life.

1. Cole, H. H., *Am. J. Anat.*, 1936, v59, 299.
2. Hamburger, C., *Acta Endocrinol.*, 1957, v24, Suppl. 31, 59.
3. Williams, P. C., *J. Endocrinol.*, 1945, v4, 137.
4. Zarrow, M. X., Caldwell, A. L., Hafez, E. S., Pincus, G., *Endocrinol.*, 1958, v63, 748.
5. Rowlands, I. W., *Nature*, 1942, v150, 267.
6. Everett, J. W., Sawyer, C. H., *Endocrinol.*, 1950, v47, 198.
7. Purshottam, N., Mason, M. M., Pincus, G., *Fert. and Ster.*, 1961, v12, 346.

Received April 17, 1962. P.S.E.B.M., 1962, v110.

Effect of Malnutrition on Liver Copper in the Rat. (27512)

W. D. ALLEN AND H. SCHNIEDEN* (Introduced by E. F. Adolph)
Department of Pharmacology, University College, Ibadan, Nigeria

Low plasma copper levels have been reported in malnourished infants and in kwashiorkor(1,2,3). The latter disease is associated with a protein carbohydrate dietary imbalance and it is possible experimentally to produce a syndrome in rats similar to kwashiorkor, by feeding animals a low protein high carbohydrate diet(4). It therefore seemed of interest to see what effects such a "kwashiorkor-like" diet would have on the liver copper of malnourished rats.

Methods. Rats of Wistar strain and with initial weight between 40-73 g were randomly divided into 2 groups. One group was placed on diet 41B(5) while the other was placed on a low protein diet which consisted of cassava plus vitamin and mineral supplements(6).

The animals were killed between the 25-27th day and their livers were perfused with Ringer Locke solution to remove blood, using the technic described by Deegan and Maegraith(7). The livers were then weighed, dried, reweighed and homogenized. Aliquots of the homogenate were taken for estimation of copper using the method of Williams and Morgan(8). In general quadruplicate estimations were done and the mean results of the 4 estimations noted. In some instances due to small size of the liver only duplicate estimations were done.

Results. Table I shows that animals kept on a standard rat cube diet gained weight while those on the cassava diet lost weight. The total liver copper was significantly different for both the groups. However there was no significant change in the liver copper/100 g liver or in the liver copper/g body weight.

Discussion. The finding shows that in these animals the fall in liver copper is correlated with fall in liver weight and body weight. It is probable however, since changes in body weight may reflect loss of tissue and possible gain in fluid leading to oedema, that in the later stages of malnutrition this corre-

TABLE I. Effect of Feeding Diet 41B or Cassava Diet on Liver Copper. (Mean ± S.E. of 20 animals on cassava diet, and 18 animals on control diet.)

Initial wt (g)	% wt change	Total liver copper, μg	Copper, μg/100 g liver	Copper, μg/g body wt
53.7 ± 1.9	+127.3 ± 14.2	28.2 ± 1.8	544.5 ± 42.0	.26 ± .05
48.5 ± 2.2	− 27.8 ± 7.3	8.2 ± .8	547.2 ± 94.6	.21 ± .06
Not significant*	Significant	Significant	Not significant	Not significant

* Considered significant if $P < 0.01$.

* Present address: Inst. of Neurology, Queen's Square, London, W.C.1.

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