that the concentration of fats in the liver of untreated nephrotic animals is significantly diminished when plasma lipids are markedly increased, lends support to the hypothesis (8) that the nephrotic hyperlipemia may be due to an inability of the liver to take up plasma lipids. It has been proposed(9) that ethionine interferes with protein formation. Since practically all serum lipids are present as lipoproteins, it is conceivable that an effect of ethionine on lipoproteins in the liver explains both the increase of liver fat and concomitant decrease of plasma lipids in nephrotic rats.

It is of interest to note that the abolition of the nephrotic hyperlipemia remained without demonstrable effect on the course of the nephrotoxic renal disease in rats.

Summary. 1. Dl-ethionine reduces the markedly increased plasma lipid concentration of nephrotic rats to normal or subnormal values within 3-5 days. 2. The diminished liver fat values regularly noted in nephrotic rats increase to normal levels under ethionine administration while carcass fat values remain unchanged. 3. The effect of ethionine on hyperlipemia and liver fat of nephrotic rats was noted in female as well as male rats, and was not prevented by methionine. 4. These results lend support to the view that the nephrotic hyperlipemia may be due to an inability of the liver to take up plasma lipids normally.

1. Heymann, W., and Lund, H. Z., *Pediatrics,* 1951, v7, 691.

2. Feinberg, H., Rubin, L., Hill, R., Enterman, C., and Chaikoff, I. L., *Science,* 1954, v120, 318.

3. Payne, R. W., *Endocrinology,* 1949, v45, 305.

4. Heymann, W., and Hackel, D. B., to be published.

5. Farber, E., Simpson, M. V., and Tarver, H., *J. Biol. Chem.,* 1950, v182, 91.

6. Jensen, D., Chaikoff, I. L., and Tarver, H., *ibid.,* 1951, v192, 395.

7. Heymann, W., and Hackel, D. B., *Metabolism,* 1955, v4, 258.

8. Rosenman, R. H., Friedman, M., and Byers, S. O., *J. Clin. Invest.,* 1955, v34, 700.

9. Simpson, M. V., Farber, E., and Tarver, H., *J. Biol. Chem.,* 1950, v182, 81.

Received December 2, 1955. P.S.E.B.M., 1956, v92.

Effect of Pre-Treatment of $P^{32}O_4$ Solutions on Uptake and Release of P^{32} by Erythrocytes.* (22379)

JOHN M. GINSKI,† JOHN F. THOMSON, AND AKIRA OMACHI.
Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill., and Department of Physiology, Stritch School of Medicine, Loyola University, Chicago.

The presence of non-orthophosphate contaminants in $P^{32}O_4$ solutions has been recognized by several investigators. Causey and Harris(1), studying the uptake of $P^{32}O_4$ by skeletal muscle, attributed some of their erratic results to strongly adsorbable trace substances, and suggested that evaporation to dryness with acid reduced the interference by these compounds. Rouser and Neuman detected impurities in Oak Ridge $P^{32}O_4$ preparations, and recommended heating with nitric acid(2). Sato *et al.*(3) subsequently showed that these entities could be separated from orthophosphate by electrochromatography. In connection with studies on the release of phosphate from erythrocytes we were interested in learning whether we would have to use the rather tedious electrochromatographic separation to obtain a homogeneous preparation of orthoradiophosphate, or whether acid-evaporation would serve. A comparison of the two procedures in release studies gave results which prompted us to carry out further experiments on the uptake of phosphate by erythrocytes.

Methods. In the release studies, erythro-

* Work performed under the auspices of the U. S. Atomic Energy Commission.

† Present address: Department of Pharmacology, University of Chicago.

cytes from healthy adult mongrel dogs were used. To a 45 ml portion of heparinized blood, obtained by jugular puncture, were added 20 μc $H_3P^{32}O_4$, 0.2 millimole pH 7.4 phosphate buffer, and 0.25 millimole of glucose. This blood was aerated at 37°C for 2 hours. The cells were then collected by centrifugation at 3000 rpm for 10 minutes at 2°C, washed twice with fresh plasma, and resuspended in plasma. Aliquots were transferred to Warburg flasks, gassed with O_2:CO_2 or N_2:CO_2 (95:5), and placed in a water bath at 37°C where they were shaken constantly at 96 oscillations per minute. After equilibration for 10 minutes, and at frequent intervals thereafter up to one hour, samples of plasma were prepared for counting by pipetting aliquots onto aluminum discs. Radioactivity was measured on dried samples with a Model PCC-10 proportional counter (Nuclear Measurement Corporation). Those taken after the 10 minute equilibration were designated as the time-zero samples, and the amount of radioactivity present in these was subtracted from subsequent counts. Uptake studies were carried out with rabbit blood. A flask containing a 10 ml sample of heparinized blood was gassed with O_2:CO_2 and equilibrated at 31°C for 30 minutes, with constant stirring. One-tenth ml of $P^{32}O_4$ solution (2 μc) was added. Ten minutes later, and at frequent intervals thereafter, 0.7 ml of blood was withdrawn, and the plasma prepared and counted as described above. Two flasks, containing different preparations of $P^{32}O_4$, were used in each experiment. $P^{32}O_4$ solution obtained from Oak Ridge was pre-treated in 3 ways. The first consisted of evaporation to dryness on a hot plate (ca. 270°C) with HNO_3 and H_2O_2. Electrochromatography plus radioautographic analysis demonstrated that the material thus treated contained an entity which did not migrate with the orthophosphate component. The second pre-treatment consisted of an electrochromatographic separation by the method of Sato *et al.*(3). The zone containing orthophosphate was located on the filter paper by radioautography. The orthophosphate was then eluted, and in order to remove the slight amount of paper fibers, lactic acid, and metallic ions found in the eluate, treated again with HNO_3 and H_2O_2 (but not taken to complete dryness, so that the temperature of the sample was never above *ca.* 100°C), and passed through a cation exchange resin (Dowex 50). The homogeneity of this material was confirmed by radioautography. Three different batches were used in these studies. The third preparation consisted of a sample of electrochromatographically homogeneous $P^{32}O_4$ which was evaporated to dryness and heated for 30 minutes in a sand bath at 300°C, then resuspended in 0.05 N HCl. This treatment results in formation of significant amounts of polyphosphates(4). These pre-treated solutions are thus identified as (I) acid-evaporated only, (II) electrochromatographed, and (III) electrochromatographed and evaporated.

Results. The data obtained on release of phosphate in 3 experiments with electrochromatographed $P^{32}O_4$ and in 2 with acid-evaporated material are shown in Fig. 1. It is apparent that the release of phosphate was about twice as fast in those experiments in which the electrochromatographed material

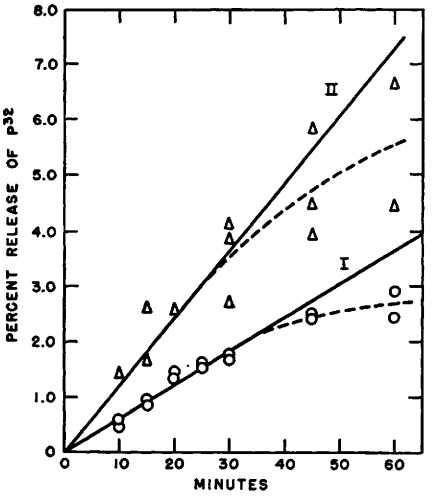

FIG. 1. Release of phosphate under aerobic conditions from dog erythrocytes previously incubated with (I) acid-evaporated and (II) electrochromatographed P^{32}. Each point is an avg of duplicate determinations. The solid lines are the slopes calculated by the method of least squares using values obtained for the first 30 min. The values are for the electrochromatographed, .123%/min., S.E. .0077; and for the acid-evaporated, .061%/min., S.E. .0018; $p < .001$.

was employed. Fig. 2 shows that the inhibition observed aerobically was noted under anaerobic conditions as well. Other experiments have been carried out at different temperatures, and similar effects have been observed; these experiments will be discussed in detail elsewhere.

The results of 3 experiments in which uptake of phosphate was measured are shown in Fig. 3. It is clear that the treatment employed here produced an inhibitory substance which decreased the initial rate of uptake by nearly 60%. As in the case of the release experiments, studies at other temperatures have invariably confirmed the observation that phosphate uptake is diminished when evaporated $P^{32}O_4$ solutions are used.

Discussion. These data emphasize that the pre-treatment given to radioactive phosphate solutions prior to use in biological systems can drastically influence experimental results. Evaporation with HNO_3–H_2O_2 either fails to remove a pre-existing inhibitor, or else produces an inhibitory substance. The latter explanation is seemingly favored by the fact that evaporation of electrochromatographically homogeneous $P^{32}O_4$ effected an inhibition in phosphate uptake, although the former possibility is of course not excluded. That the electrochromatographic treatment may have produced an activator which could be destroyed by subsequent evaporation seems highly unlikely, since the presumably "activated" samples are homogeneous while the "normal" ones (*i.e.*, acid-evaporated) are not.

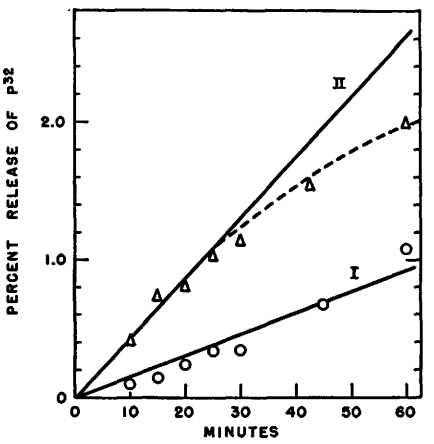

FIG. 2. Release of phosphate under anaerobic conditions, same preparations as above. The slope of II is .0439%/min., S.E. .0016; of I, .0154%/min., S.E. .0012; $p < .001$.

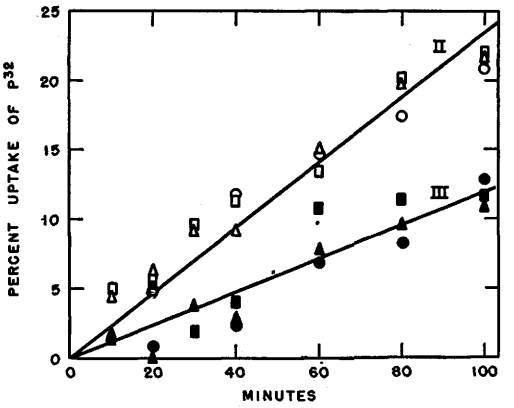

FIG. 3. Uptake of phosphate by rabbit erythrocytes incubated with (II) electrochromatographed and (III) electrochromatographed and evaporated P^{32}. The symbols show the individual experiments in which II (open symbols) and III (closed symbols) were used simultaneously. The slope of II is .234%/min., S.E. .0095; of III, .120 %/min., S.E. .0066; $p < .001$.

We have had little experience with systems other than those described here. However, one of us (A. O.) has noted that the uptake of radioactivity into the acid-soluble fraction of the isolated rabbit heart was diminished by 50% when acid-evaporated $P^{32}O_4$ was added to the perfusion fluid.

Our findings suggest that all samples of $P^{32}O_4$ be examined for homogeneity, and if evidence of heterogeneity is found the samples should be purified before use in a biological system. The electrochromatographic separation is a rather time-consuming process but appears to be a highly effective one.

Summary. The release of phosphate from dog erythrocytes was about twice as fast when blood had been previously incubated with electrochromatographed orthophosphate rather than with $P^{32}O_4$ which had been acid-evaporated without previous electrochromatographic treatment. Similar inhibitory effects were observed in the uptake of phosphate by rabbit erythrocytes when an electrochromatographed phosphate solution was evaporated to dryness prior to use.

We wish to thank T. R. Sato and W. E. Kisieleski

for their advice and assistance in the electrochromatographic and acid-evaporation technics. We are also indebted to S. A. Tyler for advice on statistical treatment of the data.

1. Causey, G., and Harris, E. J., *Biochem. J.*, 1951, v49, 176.

2. Rouser, G., and Neuman, W. F., *Fed. Proc.*, 1952, v11, 278.

3. Sato, T. R., Kisieleski, W. E., Norris, W. P., and Strain, H. H., *Anal. Chem.*, 1953, v25, 438.

4. Sato, T. R., Kisieleski, W. E., Norris, W. P., Smetana, F., and Strain, H. H., manuscript in preparation.

Received December 15, 1955. P.S.E.B.M., 1956, v92.

A Strain of Unclassified Cells Isolated from Lymph Node of Patient with "Reticulo-Endotheliosis".* (22380)

SAMUEL D. BELL, JR. AND ROBERT E. JOHNSON. (Introduced by John C. Snyder.)
(With the technical assistance of Barbara Neuman.)
Department of Microbiology, Harvard School of Public Health, Boston, Mass.

Strains of human cells *in vitro* have been found useful in cytologic and virus investigations; interest has centered mainly on epithelial-like cells either of carcinomatous origin(1,2) or from normal tissues(3), although it is well known that human fibroblasts can be subcultured for long periods of time(4). The present report describes a strain of cells derived from a lymph node biopsy of a patient with the clinical diagnosis of "reticulo-endotheliosis" and now subcultivated for 20 passages (7 months).

Clinical Data. The patient from whom this strain of cells was isolated, a 50-year-old male, noted in 1943 the gradual onset of a generalized reddish-brown, maculo-papular rash which eventually covered his entire body, except his face, and which has persisted until the present time. In May 1951, he developed symptoms of epigastric distress and weight loss; at this time positive physical findings included enlarged axillary lymph nodes and splenomegaly to the umbilicus. Peripheral blood counts were normal and biopsies of a skin lesion and lymph node revealed "nonspecific inflammatory changes." X-ray therapy (225 r) to the spleen at this time produced no change in the patient's condition. Three years later the patient noted persistent diarrhea and, in Dec. 1954, presented the typical picture of decompensated cirrhosis with muscle wasting, marked hepato-splenomegaly and ascites. Laparotomy revealed marked mesenteric lymphadenopathy with areas of infiltration into the bowel wall, liver and spleen. Pathologic studies of a mesenteric node, liver, bone marrow and skin revealed infiltration by clumps of cells which were interpreted as reticulo-endothelial cells. Since May 1955, the patient has shown a dramatic response to ACTH therapy including cessation of diarrhea, clearing of ascites, marked reduction in the size of the liver and spleen, improvement in skin lesions and disappearance of the generalized lymphadenopathy.

Materials and methods. Tissue culture methods employed were those now in wide use(5,6). A cervical lymph node biopsy was performed 6 days after start of daily ACTH therapy. Half of the node was placed in fixative for pathologic examination and half placed in synthetic medium(7), hereafter referred to as MS, for transport to the laboratory. The node was minced and placed in roller tubes both in a plasma clot and directly on the glass. The nutrient medium consisted of MS 74%, horse serum 20%, human serum 5%, chick embryo extract 1% and antibiotics, either penicillin 50 units per ml and streptomycin 50 μg per ml or tetracycline 25 μg per ml. For virus work the human se-

* This work was supported in part by research grants from the National Foundation of Infantile Paralysis and from the Research Laboratories of Chas. Pfizer Co.