zepharin and thoroughly rinsed with distilled water. The skin is then dried by blotting and rubbed off with acetone. This process degreases the skin and allows for better adhesion of the plastic disc. The chamber is cemented onto the skin by moistening the outer edge of the disc with "Duco Cement." When the rim is tacky (not fluid), it is pressed against the prepared skin site and held for 3 to 5 minutes.

After fixation of the disc, the skin outside the disc punctured with a fine gauge 1½-inch needle, the point of which is directed toward the center of the disc 0.5 ml of the diaphoric substance is injected subcutaneously (Fig. 1, 1,) with a tuberculin syringe. Local diaphoresis is rapid and copious. In 5 minutes an excess of 20 λ has usually been secreted.

When it is desired to collect the sweat, the arm is placed so the air bubble is near the center of the disc. The tip λ pipette is engaged with the vinyl plastic bottom and gentle downward pressure exerted on the disc. This causes the skin to bulge into the sweat collecting chamber and expresses the sweat into the pipette (Fig. 1, 3). It is best to slightly over fill the pipette and to remove the excess fluid from the tip by capillary action. If this is done, the tip should be wiped with a silk rag rather than filter paper or cotton which may be too highly absorbent and remove the excess too rapidly. Automatically self-filling pipettes are also available from most laboratory supply houses. The sweat is then transferred to the dilution fluid and prepared for flame photometry, or other chemical analysis. We have used the conductivity method to determine sodium. It is described in the preceding paper.

Summary and conclusion. A simplified method of collecting and measuring small quantities of rapidly secreted sweat is described. This method employs local chemical induction of sweating and the collection of this sweat in a hollowed out plastic chamber. The sweat can be transferred to a λ pipette by expressing the sweat through a capillary orifice in the chamber.

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An Antimalarial Effect *in vitro* of Parapyruvic Acid. (22093)

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When preparations of the bird malaria parasite *Plasmodium lophurae* were removed from their host duck erythrocytes and maintained extracellularly *in vitro,* their survival and development were favored by the addition to the culture medium of both adenosinetriphosphate (ATP) and sodium pyruvate (1,2). It was noted that a commercial preparation of sodium pyruvate did not have the favorable effect obtained with sodium pyruvate prepared in the laboratory(3). This fact, combined with the recent isolation by Montgomery and Webb(4) of an impurity present in some samples of sodium pyruvate, which inhibited the tricarboxylic acid cycle in isolated mitochondria, suggested an investigation of the possible antimalarial effects of this impurity. Two samples of this material, identified as a lactone of parapyruvic acid (the dimer of pyruvic acid), have been obtained through the kindness of Dr. Carmel Montgomery. They have been tested against *P. lophurae* maintained *in vitro* both extracellularly and intracellularly, as well as against the infection *in vivo.*

Materials and methods. The lactone of parapyruvic acid was dissolved in water (redistilled in a Pyrex glass still), and the solu-

tion was brought to pH 6.8 with 1 N NaOH and diluted to give a concentration of 15 mg/ml. The solution was sterilized by filtration through an ultrafine glass filter, tubed in small amounts, frozen and stored at −20°C. Freshly thawed material was appropriately diluted and used for the experiments. Since it was added to cultures having a pH of 6.8 which were subsequently held at least 1 day at 40°C, it is probable that the lactone was soon converted to parapyruvate, the form found by Montgomery and Webb to be effective in inhibiting mitochondrial respiration. For experiments with *extracellular* parasites, blood from a duckling heavily infected with *P. lophurae* was treated with guinea pig complement and an anti-duck erythrocyte serum prepared in rabbits(2). This resulted in a mixture containing free parasites, free red cell nuclei and clumps of agglutinated, mostly hemolyzed erythrocytes, both with and without parasites. A purified suspension containing only free parasites and free red cell nuclei was obtained by removing the agglutinated cells by means of a 40-second low speed centrifugation (to a maximum speed of 400 rpm). The parasite suspension was added, in amounts of 0.6 ml for purified suspensions and 0.3 ml for unfractionated suspensions, to 50 ml flasks lined with a thin plasma clot(3) and containing 3.3 ml of culture medium. The medium was a concentrated extract of duck erythrocytes prepared in the manner previously described(1) and containing all of the supplements of known chemical nature which have been found(5) to favor the extracellular survival of these intracellular parasites (yeast adenylic acid, coenzyme I, ATP, coenzyme A, malate and pyruvate). Appropriate small volumes of suitable dilutions of the stock solution of parapyruvate were added to some of the cultures. The flasks were incubated on a rocker at 40°C, and a current of air with 5% CO_2 was passed through them.

An initial Giemsa-stained film was prepared from the parasite suspension used as inoculum. After one day of incubation samples were taken from each flask and used for the preparation of Giemsa-stained films and wet mounts. The latter were examined with a phase contrast microscope(1). The stained films served for counts of the proportions of degenerate(1) parasites. For experiments with parasites developing *intracellularly* in suspensions of intact duck erythrocytes the medium and methods were those previously found to support multiplication of *P. lophurae*(6,7). Each culture flask held 6 ml of a suspension containing about 1,000,000 red cells per cu mm, with about 50 parasites per 1000 red cells. The parapyruvate was again added as small volumes of appropriate dilutions of the stock solution. The flasks were incubated on a rocker at 40°C, and a current of air with 5% CO_2 was passed through them. Giemsa-stained films were prepared from each flask initially (Day 0) and after 1 and 2 days of incubation. These were used to count the parasites per 1000 red blood cells and the proportions of parasites in different stages of development.

TABLE I. Effect of Parapyruvate on Extracellular Survival *in vitro* of *Plasmodium lophurae*. Culture medium was a duck erythrocyte extract prepared as previously described, except in Exp. A where the extract was diluted with isotonic diluent to one-third its usual strength. A purified suspension of extracellular parasites served as inoculum in Exp. A-D. In Exp. E an unpurified mixture of hemolyzed, agglutinated erythrocytes and free parasites was used.

Exp.	Flasks	Parapyruvate added, mg/ml	% degenerate parasites in stained films made after 20 hr at 40°C
A	1, 2	0	3, 2
	3, 4	.85	32, 25
B	1, 2	0	3, 2
	3, 4	.40	14, 15
C	1, 2	0	10, 9
	3, 4	.40	20, 20
	5, 6	.04	20, 23
	7, 8	.008	12, 19
D	1	0	1
	2	.4	13
	3	.04	6
	4	.008	1
E	1, 2	0	1, 1
	3, 4	.08	4, 5
	5, 6	.025	7, 4
	7, 8	.008	2, 3

Results. The deleterious effect of the parapyruvate preparation on the extracellular survival of *P. lophurae* is clearly shown by the

TABLE II. Effect of Parapyruvate on Development of *P. lophurae* in Suspensions of Duck Erythrocytes Maintained *in vitro*, in 8 Flasks.

Parapyruvate added, mg/ml	% young parasites (uninucleate, not pigmented) on day: 0	1	2	Total parasites/1000 red blood cells on day: 0	1	2
0	48	0	68	50	52	59
0	74	0	68	52	51	51
.075	52	0	24	52	50	18
.075	64	0	36	48	33	29
.025	48	2	60	48	42	20
.025	56	0	64	41	31	21
.008	72	0	68	50	43	26
.008	80	0	74	68	82	20

experiments summarized in Table I. Concentrations as low as 40 and 25 μg per ml were definitely harmful to the parasite, whereas a concentration of 8 μg per ml appeared to be at the border line of activity. The relative degrees of degeneration seen in the stained films were likewise seen in the fresh preparations examined by phase contrast.

The parapyruvate similarly had a deleterious effect on the development of the parasites within erythrocytes, as illustrated by the experiment shown in Table II. It will be seen that all 3 concentrations tested reduced the number of parasites present after 2 days' incubation to about half that of the control flasks. Some reduction in parasite numbers was already evident at the two higher concentrations after one day's incubation. It is worth noting that in the flasks with the highest concentration (75 μg/ml) the proportion of young parasites which reinvaded erythrocytes on day 2 was much less than in the control flasks or in those with lower concentrations.

Discussion. The inhibition of respiration of isolated mitochondria by parapyruvate appears to depend on competition between this compound and alpha-ketoglutarate for alpha-ketoglutaric acid oxidase(4). Since there is good evidence for the participation of the tricarboxylic acid cycle in the oxidative metabolism of malaria parasites(8), it may well be that the deleterious effect of parapyruvate on the parasites is likewise a result of interference by this compound with the utilization of alpha-ketoglutarate. In the experiments with extracellular parasites this effect occurred at concentrations down to 1.5 x 10^{-4}M in the presence of pyruvate at a concentration of 5 x 10^{-3}M. In the experiments with parasites developing intracellularly the effect was marked even at the lowest concentration tested of 5 x 10^{-5}M. Perhaps the absence of added pyruvate and the longer period of incubation in the latter experiments both contributed to this greater effect.

The ability of the parapyruvate to penetrate infected duck erythrocytes and reduce the numbers of *P. lophurae* developing intracellularly suggested that the compound might have some antimalarial action *in vivo*. This did not prove to be the case. Ducklings infected with *P. lophurae* were treated once on the 3rd day and twice on each of the 4th and 5th days of their infection with doses of 2.5 to 10 mg parapyruvate per 100 g body weight, administered in solution intravenously. No effect on either the course of the infection or the ducklings was observed. Presumably the parapyruvate was altered before it could exert its action on the malaria parasites.

Summary. When the erythrocytic forms of *Plasmodium lophurae* were maintained extracellularly *in vitro* the addition of parapyruvate at concentrations down to 25 μg per ml increased the proportion of degenerate parasites present after one day's incubation. In cultures with intracellular parasites in suspensions of intact duck erythrocytes, the addition of the parapyruvate at concentrations down to 8 μg per ml decreased sharply the numbers of parasites present after two days' incubation. Somewhat higher concentrations also interfered with the reinvasion of erythrocytes by merozoites formed in such cultures. No antimalarial effect was observed when the compound was administered intravenously to ducklings infected with *P. lophurae*.

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Paradoxical Retention of Uric Acid by Uricosuric Drugs in Low Dosage.* (22094)

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It has long been known that the renal excretion of uric acid in man is increased by large doses of salicylate and suppressed by small doses(1,2). Recent, more adequately documented studies(3) showed that acetylsalicylic acid in 5.2 g daily oral dosage caused a mean increase of 37.2% in the 24-hour urinary uric acid output of 15 subjects, with a concomitant mean fall of 25.5% in plasma uric acid, whereas 2.0 g daily dosage resulted in a mean decline of 14.4% in the urinary excretion and 7.5% rise in the plasma concentration of uric acid. Renal clearance studies indicate that this reversible effect is related to the concentration of salicylate in blood plasma(4) or, more precisely, in the urine(3); high salicylate concentrations cause an increase in the renal clearance ratio, $C_{\text{uric acid}}/C_{\text{inulin}}$, low salicylate levels depress this ratio.

Our data illustrate these relationships in respect to salicylate and indicate further that the reversibility of effect on uric acid clearance can be demonstrated, by appropriately designed experiments, to occur with all other uricosuric drugs we have examined, namely, phenylbutazone, phenylbutazone analogs, and probenecid. It would appear that the capacity to cause uric acid retention when given in low dosage is, to greater or lesser degree, a property common to uricosuric agents. The observation may have implications of interest in relation to the still obscure nature of renal tubular transport systems for uric acid.

Methods. The experiments were performed in gouty subjects in the intercritical phase of the disorder. Standard renal clearance technics(5) were employed throughout, with minor modifications described elsewhere(6). The general experimental design was to obtain 3 or 4 pre-medication urine collection periods for estimation of the inulin and PAH clearances, then to infuse the drug to be studied at a slow rate yielding low initial plasma drug concentrations, and gradually to increase the rate of drug infusion to attain uricosuric plasma drug levels. The 6-10 urine collection (catheterization) periods obtained during infusion of the drug in each experiment were each of 10-30 minutes duration; urine samples (spontaneous voidings) collected in some instances after termination of the drug infusion represented 2-hour intervals during the day and 4-hour intervals at night. Blood samples were procured at the midpoint of each urine collection period. In the salicylate infusion experiments, 4 g sodium salicylate dissolved in 500 ml isotonic saline solution was injected at a rate of 12-15 mg salicylate/min. for one or more periods, with an increment of 5-10 mg/min. in each urine collection period thereafter. In 2 phenylbutazone experiments, the infusing solution contained 2 g phenylbutazone/250 ml isotonic saline solution, was delivered at a rate of 12 mg/min. for 2 clearance periods and at double the rate thereafter. In the 3 remaining phenylbutazone, the 2 G-25671 and 6 of the 8 phenylbut-

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