anovulatory cycle. Hartman(8) has reported that the monkey ovulates infrequently during the summer months, and the menstruation occurred toward the end of May. If the cycle was in fact anovulatory, the source of the progesterone is unknown. The high level of progesterone found in the second cycle in this animal indicates that a corpus luteum must have been present. This cycle, however, was 32 days in length, and menstruation began on June 26, 14 days after the last assay. Consequently, the level of progesterone in relation to the onset of the second menstruation is unknown.

Single values in 3 other intact monkeys may be mentioned. In one animal a level between 7.7 and 8.9 μg per ml plasma was found 10 days before the onset of menstruation; in a second animal the level was 2.8 μg per ml 3 days before menstruation; in a third animal the level was 0.9 μg per ml 2 days before menstruation. It is clearly impossible to say whether these isolated values conform to any pattern shown by the 2 animals studied throughout a cycle. The values for bound plasma progesterone ranged from less than 0.2 to 0.4 μg per ml. The small fluctuations encountered seemed to be quite unrelated to the phase of the cycle

The uniformly low levels of bound progesterone are consistent with our other experience with this fraction. Its level has been low under all physiologic circumstances in several species(6). It has been high only in animals receiving injections or carrying pellets of progesterone; in the mouse the bound fraction was biologically inert, and binding was a means of hepatic inactivation of progesterone(9).

Summary. Bioassay of the free and bound plasma progesterone at various intervals through a menstrual cycle in each of 2 monkeys indicate the level of free progesterone to be low during and following menstruation, high near the middle of the cycle, lower following this peak, and high in the latter half of the cycle. A low level was present at or preceding menstruation. The levels of the bound fraction were low at all times.

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Survival of *Schistosoma mansoni in vitro*.* (17617)

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Information about the survival of schistosomes *in vitro* has been limited to the observation that *Schistosoma japonicum* can be maintained alive in serum for periods of 8 to 83 days(1,2). Similarly, we have found that *Schistosoma mansoni* survives for 14 to 18 days in horse serum. Knowledge about the nutritional requirements of these trematodes could be acquired by investigating the constituents of serum, essential for their survival. Accordingly, studies designed to define these factors were initiated using *Schistosoma mansoni*.

Methods. All experiments were conducted under aseptic conditions. Pairs of schistosomes

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were removed, with dissecting needles, from the mesenteric and portal veins of mice infected 6 to 8 weeks previously by intraperitoneal injection of 160 cercariae per mouse. The helminths were washed in 5 to 10 ml of a medium which had the same composition as that used in the subsequent experiment. The parasites were transferred to Stender dishes containing, per pair of worms, 3 to 5 ml of the medium to be tested; incubation was carried out at 37°C. The medium was renewed every 3 days by replacement rather than by transfer of the schistosomes. Motility of the worms was used as the criterion of survival. Usually, this was easily detected. In questionable cases, the worms were considered dead if motility was absent in two separate observation periods (5 minutes), under the low power dissecting microscope. The chemically defined medium (SM), had the following composition per 100 ml: NaCl, 753 mg %; $CaCl_2$, 4.4 mg%; $NaNO_3$, 0.10 mg %; Na_2SO_4, 0.66 mg %; KCl, 1.73 mg %; $MgCl_2$, 50 mg %; $MnCl_2$, 5.4 mg %; $FeCl_2$, 0.04 mg %; tris(hydroxymethyl)aminomethane buffer (pH 8.0), 0.0025M; $NaHCO_3$, 0.0052M; Na orthophosphate buffer (pH 8.0), 0.0021M; DL-lysine, 7.8 mg %; DL-methionine, 6.5 mg %; DL-threonine, 6.5 mg %; DL-valine, 6.5 mg %; L-arginine HCl, 3.95 mg %; L-histidine HCl, 1.3 mg %; DL-isoleucine, 5.2 mg %; DL-phenylalanine, 2.5 mg %; L-leucine, 7.8 mg %; DL-tryptophane, 2.0 mg %; DL-alanine, 10 mg %; glutathione, 10 mg %; cysteine HCl, 0.10 mg %; p-aminobenzoic acid, 0.125 mg %; carotene, 0.01 mg %; ascorbic acid, 0.01 mg %; thiamine, 0.01 mg %; riboflavin, 0.02 mg %; niacin, 0.05 mg %; pyridoxine HCl, 0.10 mg %; inositol, 0.05 mg %; Ca pantothenate, 0.10 mg %; biotin, 0.0005 mg %; choline chloride, 0.05 mg %; folic acid, 0.0001 mg %; uracil 2 mg %; adenine sulfate, 2 mg %; xanthine, 2 mg %; hypoxanthine, 2 mg %; guanine, 0.4 mg %; glucose, 50 mg %. In this medium the schistosomes survived for only 12 to 18 hours.

Results. The schistosomes survived for almost the same length of time in serum ultrafiltrate (UF) (10 to 12 days) as in whole serum (14 to 18 days). This indicates that protein and other non-dialyzable components of serum are of little significance to the survival of the worms. The pH of individual batches of serum ultrafiltrate varied considerably (7.8 to 9.5). Accordingly, attempts were made to stabilize the pH of the UF. The CO_2 contained in the UF was driven off by aeration for 10 minutes at pH 4.0. The pH was then adjusted to 8.0 with NaOH. This solution was sterilized by filtration through a sintered glass filter (Corning Glass No. 330990). One ml of 0.5M $NaHCO_3$ and 1.25 ml of 0.2M tris(hydroxymethyl)aminomethane buffer (pH 8.0)(3) per 100 ml were added to this serum ultrafiltrate immediately before each experiment. No difference in the duration of survival of the worms was observed regardless of whether they were incubated in untreated UF or in UF treated in this manner. A twofold increase in the bicarbonate concentration reduced the survival time. Orthophosphate (0.005M) and pyrophosphate (0.005M) buffers proved highly toxic, allowing survival for not more than one day. The brief survival of the worms in SM was due to a nutritional inadequacy rather than to a toxic effect, since addition of all the constituents of this medium (with the exception of NaCl) to UF, in concentrations similar to those used in SM, did not shorten the period of survival of the organisms. However, sodium nitrate and sodium sulfate in a concentration of 5 mg % proved toxic to the worms. Addition to SM of the following materials failed to increase the period of survival of the worms: ashed ultrafiltrate, $MnCl_2$, glutamine, acid or enzymatic casein hydrolysates, ornithine, creatine, creatinine, urea, Tween 80, beta-hy-

TABLE I.
Survival of *S. mansoni* in SM Supplemented with Protogen.

Protogen units per ml	Survival in days
2000	9
90	4
60	1
30	1
15	<1

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TABLE II.
Survival of *Schistosoma mansoni* and *Litomosoides carinii* Under Aerobic and Anaerobic Conditions.

Helminth	Medium	Atmosphere	NaCN	Survival (days)
S. mansoni	UF	Air	—	12
" "	UF	100% N_2	—	5
" "	UF	Air	1×10^{-3} M	6
" "	Ox Serum	"	—	16
" "	" "	100% N_2	—	5
Litomosoides carinii	" "	Air	—	7
" "	" "	100% N_2	—	< 1

droxybutyric acid, citrate, ribose, xylose, ribonucleic acid, desoxyribonucleic acid, a concentrate of strepogenin,† vitamin B_{12}, a concentrate of animal protein factor,‡ thymine, pyridoxal. In a medium containing 50% UF and the same concentrations of NaCl and glucose as are present in SM, survival was as long as in undiluted UF. However, a further reduction of UF to a concentration of 25% permitted survival of the worms for only 3 days instead of 11 days. Addition of an aqueous extract of muscle (Difco No. B126) to SM resulted in a marked increase in survival time. If SM contained 25 mg/ml of this extract, survival of the worms almost equalled that observed with undiluted ultrafiltrate. The factor or factors in this muscle extract responsible for survival were found to be soluble in 35% and insoluble in 90% ethyl alcohol. A significant increase in the periods of survival occurred also when a fraction of purified protogen (4,5) (NP-41-160-1-H-0)‡ was added to SM (Table I).

S. mansoni can survive in the host under conditions that produce a marked reduction in the respiratory metabolism of the fluke (6,7). Furthermore, this parasite utilizes little or no energy liberated from oxidative metabolism (8). Additional evidence that respiratory metabolism is of minor significance to survival is supplied by the fact that the schistosomes can survive (Table II) in 100% nitrogen for at least 5 days. Similarly, survival for 5 days occurred in 1×10^{-3}M cyanide (under conditions where loss of HCN was precluded). A complete inhibition of respiration was observed in worms which had been incubated for 4 days in cyanide 1×10^{-3}M), while their rate of aerobic and anaerobic glycolysis was the same as in controls not exposed to cyanide. Since in similar media, survival is more than twice as long aerobically as anaerobically, a critical requirement for oxidative metabolism must develop during or at the end of 5 days. By contrast, the filarial worm, *Litomosoides carinii*, did not survive under anaerobic conditions for more than 12 hours. This helminth has a high requirement for oxidative metabolism and partial inhibition of its oxygen uptake in the host results in its death (9,10).

Summary. 1. A method is described for studying the survival of *S. mansoni in vitro*. 2. These parasites survived in serum for 14 to 18 days; in serum ultrafiltrate for 10 to 12 days. 3. In a chemically defined medium, the survival of the parasite did not exceed 18 hours. 4. Addition of an aqueous extract of beef muscle or of a purified fraction of protogen to the synthetic medium significant-

† Kindly supplied by Dr. D. W. Woolley.

‡ Kindly supplied by Drs. T. H. Jukes and E. L. R. Stokstad, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

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ly increased the period of survival. 5. Under completely anaerobic conditions the schistosomes survived for 5 days.

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Changes in Carcass Urea Following Evisceration in the Rat. (17618)

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The generally accepted conclusion that liver is the only mammalian tissue that can synthesize urea is based in major part upon the classical studies of Bollman, Mann and Magath(1) who noted that the level of blood urea remained steady following hepatectomy-nephrectomy in the dog, of Bollman and Mann(2) who found no evidence for the conversion of ammonia to urea in the liverless dog, and of Krebs and Henseleit(3) who found that the liver slice was the only tissue which could convert ammonia into urea. Maddock and Svedberg(4) found no evidence for urea synthesis in the liverless monkey. Observations upon the liverless animal have been limited to a few species and to survival periods of a few hours. We have studied changes in the blood and tissues of eviscerate rats that survive for periods of 48 hours and longer. Ingle, Prestrud and Nezamis(5) noted that the blood urea of the rat infused with solutions of glucose and insulin did not fall during the first 24 hours following evisceration although the kidney continued to excrete urea and the animal was in positive water balance, both of which might be expected to lower the urea of the body-water in the absence of urea synthesis. Since the circulation and water metabolism of the eviscerate rat are greatly changed from normal, it would be unsafe to assume that the urea content of its blood is representative of its distribution in the body water or that these observations on the rat provide valid evidence for the extra-hepatic synthesis of urea.

We have reinvestigated the problem by determining the urea content of the carcass and urines of rats at the time of evisceration and of similar rats 24 and 48 hours following evisceration. There was a decrease in the total urea of the carcass and urine following evisceration.

Methods. Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At attainment of a weight of 185 to 205 g, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. When the animals reached a weight of 250 ± 2 g they were anesthetized with cyclopal and eviscerated by the method of Ingle(6). Asepsis was preserved in both stages of the operation. A solution containing glucose, insulin, and 0.9% sodium chloride was infused into the saphenous vein of the right hind leg by means of a constant injection machine which delivered fluid from each of 6 syringes at the rate of 20 cc in 24 hours. The glucose load was 44 mg per 100 grams of rat per hour and 4 units of regular insulin (Lilly) per rat per 24 hours. Temperature was constant at 26.5°C.

The total urea of the carcass was determined as follows: The tail of the eviscerate rat was cut into small pieces and placed in

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