during the 9-day culture period. 2. The average percentage of successful grafts is increased by implantation two or more pieces of tumor within a single egg, and by incubating the excised tumor for several hours in rabbit serum before implantation onto the chorioallantois. 3. The tumor does not metastasize from the chorioallantois in spite of apparent direct contact between the embryonic blood and the tumor cells.

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Use of Antipyrine in Measurement of Total Body Water in Animals.* (18050)

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A method for determining total body water in man by means of antipyrine has recently been described(1). In a series of tests this drug fulfilled the characteristics a substance should possess for the measurement of total body water in man: It was not toxic in the amounts necessary for the determination, it was distributed evenly and rapidly throughout the body water, it was degraded and excreted at a regular rate, and the analytical procedure for its determination was accurate and simple.

Most methods for measuring total body water are based on the volume of distribution of a foreign substance after intravenous administration. These procedures are in the main unsatisfactory because of unequal distribution in the tissue water of the body. In contrast, determining total body water with deuterium oxide or tritium is more satisfactory as these isotopes distribute evenly, but the analytical technics are difficult and the cost high(2,3,4).

Antipyrine is rapidly transformed in the dog and it was thought that this characteristic would preclude its use for the measurement of total body water. The following studies were carried out to ascertain whether or not antipyrine is in fact satisfactory for this purpose.

Methods. Measurement of antipyrine in tissues and plasma has been described‡(5). Following intravenous injection, the distribution of antipyrine was determined in representative tissues of one rabbit at 50 minutes following injection and 2 dogs at 1½ hours and 2¾ hours respectively. The animals were killed by intravenous air injection and the tissues were analyzed immediately.

The following technic was employed to measure total body water: control sample of blood was withdrawn to determine the plasma blank value for correcting subsequent samples; 75 mg of antipyrine per kilo body weight in the form of a 5% solution in distilled water was injected intravenously from a burette or calibrated syringe; blood samples were withdrawn and heparinized at 2, 3, and 4 hours after injection; plasma and cells were

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‡ The method is based on the addition of sodium nitrite to a plasma filtrate or tissue extract with the resulting formation of a 4-nitroso antipyrine which can be read in the spectrophotometer at 350 mu. Appropriate dilutions of the plasma are made so that readings fall in the significant colorimeter range.

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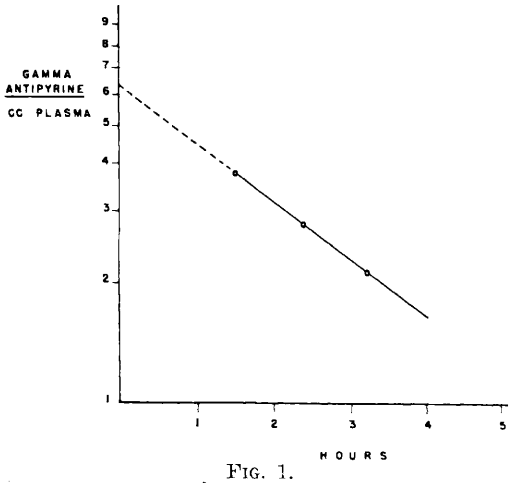

FIG. 1.

Plasma levels of antipyrine. The curve for the plasma levels is extrapolated to zero time to correct for the metabolism of the drug during the time required for uniform distribution.

separated after centrifugation and the plasma stored in stoppered tubes for subsequent antipyrine analysis.

The plasma concentration at zero time (the concentration at the time of injection, if uniform distribution had been instantaneous and if none of the substance had been metabolized) was calculated by plotting the plasma levels on semilogarithmic paper and extrapolating the straight portion of the time-concentration curve (2nd, 3rd and 4th hours) back to the time of injection (Fig. 1).

The plasma water level of antipyrine was calculated by dividing the plasma level of antipyrine by the water content of the plasma (.93 average).

The calculation for total body water was made as follows:

$$\text{Body water (liters)} = \frac{\text{amount of drug injected (mg)}}{\text{plasma water level (mg per liter)}}$$

To obtain a comparison of antipyrine and desiccation methods, 5 monkeys, 4 dogs, and 4 rabbits were used. After total body water had been determined with antipyrine the animals were weighed and sacrificed. The entire animal was ground, and the final mass then spread over pans and placed in an oven at a temperature of 95°C for a period of 4 to 7 days until constant weight was achieved.

Results. The ratio of tissue water antipyrine concentration divided by plasma water antipyrine concentration was found to be close to unity in all tissues, with three exceptions. These were in the liver and kidney where degradation and excretion were presumably taking place, and in the lung. The antipyrine concentration in tissues appeared to be related to their water content (Table I).

Values for total body water in 4 dogs, 5 monkeys, and 4 rabbits obtained by antipyrine and by desiccation did not differ significantly (Table II). The total body water in terms of per cent body weight ranged from 62.8% to 77.8% with an average of 70.9%. The average difference was 54.5 cc, with values ranging from 10 to 122 cc. The coefficient of correlation was 0.93.

After plotting the plasma concentration as a semilogarithmic function of time, it was observed that in all species tested, a linear relationship was obtained within two hours after the injection of antipyrine. The smaller the animal, the earlier even distribution was achieved; *e.g.* in the small rabbits, even distribution was achieved after 50 minutes.

In an effort to ascertain whether the rate of transformation of antipyrine was regular when even distribution had been achieved (for extrapolation purposes), 75 mg of antipyrine per kilo of body weight was administered intravenously to 20 dogs, 5 monkeys, and 4 rabbits. Blood samples were taken at intervals following complete distribution and

TABLE I.

Distribution of Antipyrine in Water of Animal Tissues.

	Ratio $\frac{\text{tissue water antipyrine}}{\text{plasma water antipyrine}}$		
Tissue	Dog 1	Dog 2	Rabbit
Plasma	1.00	1.00	1.00
Heart	1.07	1.04	.96
Muscle	1.05	0.94	.99
Muscle	1.05	1.00	
Spleen	1.03		
Kidney	1.12	1.12	.97
Liver	1.04	1.10	1.08
Lung	0.83	0.86	1.01
Brain	0.99		
Cerebrospinal fluid	0.88	0.99	
Average	1.06	1.01	1.00

TABLE II.
Comparison of Total Body Water in Animals Determined by Antipyrine and Desiccation.

Animal	Weight (g)	Total water: Antipyrine (cc)	Total water: Antipyrine (% body wt)	Total water: Desiccation (cc)	Total water: Desiccation (% body wt)	Difference in (%)
1. Monkey	3637	2290	62.8	2412	66.4	—3.6
2. "	3321	2360	71.0	2317	69.8	+1.2
3. "	2854	1980	69.4	2046	71.6	—2.2
4. "	3496	2350	67.2	2443	69.9	—2.7
5. "	3099	2230	72.1	2146	69.4	+2.7
6. Dog	875	662	75.7	623	71.3	+4.4
7. "	1303	832	63.9	886	68.1	—4.2
8. "	3279	2272	69.5	2373	72.5	—3.0
9. "	2891	2139	73.9	2097	72.5	+1.4
10. Rabbit	1782	1330	74.6	1350	75.8	—1.2
11. "	1846	1270	68.8	1285	69.6	—0.8
12. "	660	501	75.8	481	72.9	+2.9
13. "	1172	912	77.8	902	77.0	+0.8

the plasma analyzed for antipyrine. Results indicated that the average fall in blood level due to degradation and excretion was 30% per hour and varied from 20 to 50%. However, in a given animal the rate was constant over periods up to 7 hours. Thus, the transformation of antipyrine as represented by the falling plasma level could be corrected by extrapolation of the semilogarithmic curve of the plasma concentration to zero time.

Antipyrine was found to be non-toxic in 30 dogs, 10 rabbits and 8 monkeys given 75 mg of antipyrine per kilo body weight intravenously, with the exceptions that 5 dogs exhibited excessive salivation and one dog vomited immediately following a rapid injection. It was thought that the vomiting could have been avoided by a slower rate of injection.

Discussion. It was recently shown that the total body water content in adult humans as determined by antipyrine and deuterium ranged from 40 to 60% of body weight(1). The values of total body water showed considerable variability and suggested a clear inverse relationship between the amounts of total body water and total body fat. Higher values for total body water of 65 to 70% body weight were obtained with antipyrine on trained lean individuals(6). These values were confirmed by calculating total water from specific gravity determinations done on these same subjects, assuming that fat free tissue is 71.8% water.

The average value for total body water in the animals of this series was 70.9% body weight, ranging from 62.8 to 77.8% body weight. It should be emphasized that these animals were young and lean, purposely selected in order to facilitate desiccation. In contrast, the total body water determined in ten adult dogs in connection with other experiments ranged from 53.2 to 65.8% of the body weight with a mean of 58.5%. Thus, the values of total water above 71.8% body weight indicated that the assumption that 71.8% of the fat free tissue is water is invalid. The discrepancy may be due to differences in bone density and structure not taken into account in the calculation of total body water from specific gravity determinations.

Although the rate of transformation of antipyrine in animals is relatively rapid as compared to humans, this factor does not interfere with determination of total body water with antipyrine since it has been shown that the rate of transformation is constant over the period of time necessary for the determination.

Summary. Antipyrine is distributed evenly in the various tissues of animals in close proportion to their water content. The values obtained for total body water by antipyrine and by desiccation agreed well and gave a correlation coefficient of 0.93. The rate of

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transformation and excretion of antipyrine as determined by plasma analysis was constant over a period of time sufficient for analysis.

Ease of analysis and lack of toxicity make antipyrine a suitable substance for use in measurement of total body water in animals.

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Infection and Immunity in Offspring of Mice Inoculated during Gestation with Murine Poliomyelitis Virus. (18051)

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A study of 100 pregnant mice, inoculated at various intervals during gestation with Col. SK murine poliomyelitis virus, has shown that there is a progressive increase in susceptibility to infection which begins with the fourth day of pregnancy and reaches a maximum during the last 4 days before parturition (1). The present paper deals with observations on the young from the inoculated mothers, the data being concerned with the infection of the fetus and the immunity of surviving offspring.

Materials and Methods. Among 100 gestating mice orally inoculated with Col.SK virus pregnancy terminated in 5 different ways (Table I). In addition to surviving young from these infected mothers, 6 litters whose mothers had been infected soon after parturition, another 6 litters produced by breeding mice with known immunity, and 4 normal litters contributed to the study of immunity in young mice.

Infection in the young. Fetal tissues, and tissues from offspring of mothers which had succumbed to infection after parturition were tested for recovery of virus by intracerebral inoculation of young mice.

Immunity of offspring. Litters were examined for their immunity at various intervals between weaning and 44 days of age by intranasal inoculation of Col.SK virus diluted 10^{-2}. Older littermates were tested by intraperitoneal inoculation of virus diluted 0.5 x 10^{-2}, a challenge which served also in investigating the development of maternal immunity. Surviving offspring of paralyzed mothers were placed with litters of other lactating mice, some of which were normal, whereas others were surviving experimental animals. When it became apparent that immunity in these fostered infants corresponded with the immunity of the fostering mother, the influence of suckling with immune mice was further investigated by means of interchanging normal baby mice and the young of immune mice during the 21-day period of nursing, and subsequently testing the fostered mice for their ability to resist infection.

In vitro test of milk for its capacity to neutralize virus. Milk collected(2) from 2 immune mothers was pooled and examined at various dilutions for its capacity to neutralize Col.SK virus. Pooled milk from 2 normal lactating mice served as control. Equal mixtures of milk and suspensions of virus were incubated at 37°C for 1 hour, placed overnight in the refrigerator, and then inoculated intraperitoneally into young mice.

Results. Outcome of pregnancy. (Table I). Eighteen viable litters, born to 100 female mice orally inoculated with Col.SK virus during gestation, were apparently normal at birth. One half of the litters were reared by their own mothers which had resisted infection. The remaining 9 litters came from mothers which became paralyzed after parturition; 4 of these litters were successfully reared by foster mothers. The rate of abortion was high among mice infected

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