row picture and the changes in anti-hyaluronidase titre. (6) The height of the anti-hyaluronidase titre appears to be related to the quantity of tumor tissue rather than to its invasiveness.

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Heinz Body Phenomenon in Monkey Erythrocytes, a Quantitative Method. (18449)

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Webster(1) has published an excellent review on the Heinz Body Phenomenon in Erythrocytes. In this he states that "further work on the relationship between Heinz body occurrence and the action of the spleen. . . . appears to be necessary in order to extend our knowledge of the role played by the spleen in hemolytic anemias produced by chemical agents." It has been observed that the amount of quinine hydrochloride necessary to produce the acute death of the monkey was less in animals with a severe anemia resulting from malaria than it was in monkeys with an equally severe anemia produced by phenylhydrazine(2).

Several methods have been devised for the demonstration and estimation of Heinz bodies. Cruz(3) and others(4-6) have made use of the turbidity which occurs in the blood to estimate quantitatively the amount of Heinz bodies present. Counts may be done either on fixed stained smears or on supravital preparations(7,8). Fertman and Doan(9) say, "The inclusions could be seen in unstained living blood films as refractile yellow tinted bodies. In dark field examination they appeared highly refractile, globular and irregular, similar in general appearance and distribution to the inclusions seen in bright field." Figge(10) thinks that Heinz bodies can be readily demonstrated in plain dried blood films examined with a high power objective; he found that they disappeared when examined under oil, balsam or other mounting media. Utilizing the fact that Heinz bodies are not hemolysed in distilled water, we have demonstrated that the number of Heinz bodies in the blood of the monkey may be determined by diluting the blood with water in a standard red cell pipette and counting the number of Heinz bodies on a haemocytometer. Observations were made in both the normal and splenectomized monkey.

Methods and materials. Ten *M. mulatta* monkeys, weighing from 2.6 to 4.0 kg, were used. Blood for the erythrocyte counts, smears and Heinz bodies was obtained from the marginal vein of the ears. Smears were stained with a combination of Wright's and Giemsa's stains. The number of Heinz bodies free in these smears was counted per 500 erythrocytes. Red cell counts were made using standard technics and Heinz body counts were carried out by a similar technic. The blood for the latter was drawn to the 0.5 mark on a red cell diluting pipette and the diluting fluid was drawn to the 101 mark, thus giving the count as the number of Heinz bodies in M per cmm. Distilled water and a solution of phloxine dye were used as the

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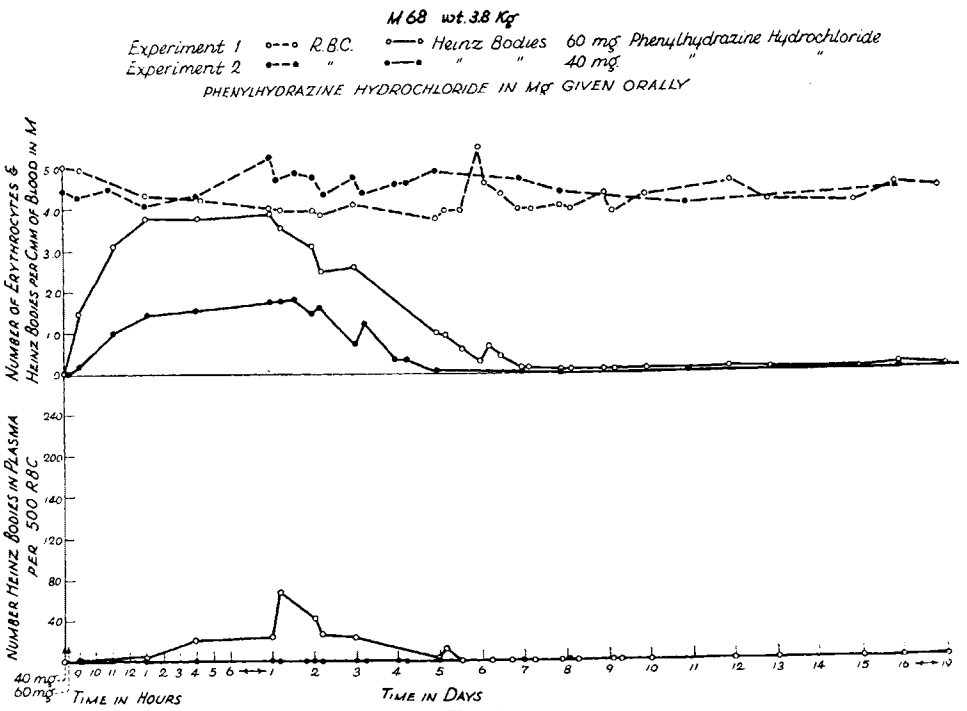

FIG. 1.

In Exp. 1, 60 mg of phenylhydrazine were given. The total erythrocyte count decreased approximately one million cells, however, the number gradually increased to approximately a normal level by the 20th day. The number of Heinz bodies as demonstrated by diluting the blood in distilled water rapidly increased during the first 24 hours of the experiment and then progressively decreased until the 8th day. The number of Heinz bodies free in the plasma was less than in the distilled water preparation. In Exp. 2, a smaller amount (40 mg) of phenylhydrazine was given. A corresponding decrease is reflected in the number of erythrocytes and Heinz bodies. In fact, there were too few to count per 500 erythrocytes during the time of the 2nd experiment. The time in hours shows that the experiment was begun between 8 and 9 A.M.

diluent. The phloxine solution was made from a stock solution in which 250 mg of phloxine powder were added to 500 ml of distilled water. A fresh solution was prepared using 5 ml of the stock solution in 200 ml of distilled water for each experiment. This dye was used to stain the Heinz bodies lightly. The blood in the pipette was shaken to assure mixing of the cells, then it was immediately placed upon a haemocytometer in the same manner as that used for an erythrocyte count. The Heinz bodies were allowed to settle on the counting chamber for 10 to 15 minutes before they were counted. The phenylhydrazine hydrochloride solution given to the monkeys was prepared by dissolving one gram of the drug in 50 ml of 95% ethyl alcohol. It was necessary to apply a little heat to dissolve the crystals. Although the preparation became deep yellow in color after a few days at room temperature, it was satisfactory for use. The drug was given orally through a small catheter. The amount given at one time varied from 20 to 150 mg. One to 3 such doses were given during an experiment. In some of the animals given phenylhydrazine hydrochloride, the number of Heinz bodies in the blood was followed for a month, at which time the spleen was aseptically removed. The monkeys again were given phenylhydrazine and the number of Heinz bodies was counted.

Heinz bodies in normal monkeys given phenylhydrazine. Seven normal monkeys, weighing from 3.2 to 4.0 kg, were given phenylhydrazine hydrochloride in amounts varying from 40 to 150 mg. Heinz bodies were demonstrated in the blood within an

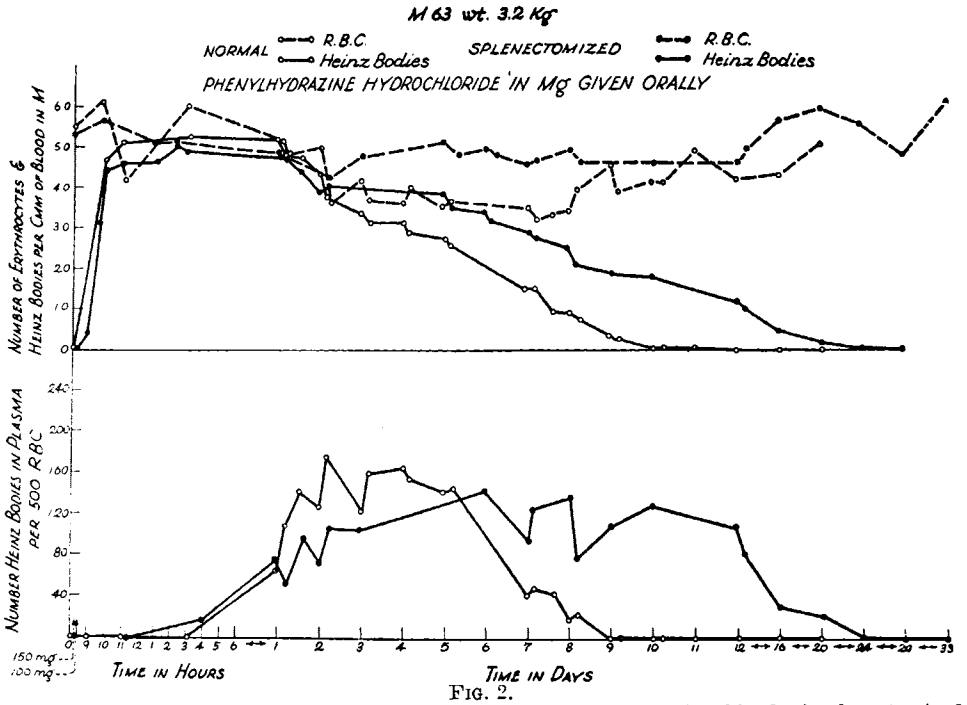

FIG. 2.

The number of Heinz bodies persists much longer in the circulating blood of splenectomized monkeys than in normal animals. The sequence of changes in the number of erythrocytes and Heinz bodies is the same as described in Fig. 1.

hour whenever the dose was 40 mg or more. The animals frequently became cyanotic after larger doses of the drug and their blood was chocolate brown in color. This color persisted for 2 or 3 days. The Heinz bodies seen within the first hour were very small and difficult to count. As time progressed, the Heinz bodies increased in size for approximately 36 hours.

Monkey 68 (Fig. 1, Exp. 1) was given 60 mg of phenylhydrazine hydrochloride. One hour later there were 1.51 million bodies per cm in the peripheral blood. Five hours later the Heinz body count was 3.89 million and the erythrocyte count was 4.30 million. Twenty-four hours later the Heinz body count was 3.94 million and erythrocyte count was 4.07 million. The number of bodies gradually decreased until the 19th experimental day, at which time none was demonstrated in the peripheral blood. The number of Heinz bodies free in the plasma during the time of this experiment is shown in Fig. 1. Seven days after Heinz bodies were last observed in Monkey 68, this animal was given 40 mg of phenylhydrazine hydrochloride (Fig. 1, Exp. 2). One hour later only 0.19 million Heinz bodies were present. The count reached its maximum of 1.86 million 32 hours later. The erythrocyte count at this time was 4.85 million. The number of Heinz bodies gradually decreased and the number of erythrocytes remained constant until the 7th experimental day, at which ime there were no Heinz bodies present. Although an occasional Heinz body could be seen within an erythrocyte in a blood smear, the number of bodies free in the plasma was too few to count per 500 red cells at any time during this 2nd experiment.

Monkeys 62 and 63 weighed 3.3 and 3.2 kg. They were given 150 mg of phenylhydrazine hydrochloride orally. The observations on Monkey 63 are shown in Fig. 2. Two hours after the phenylhydrazine was given, the Heinz body counts were 4.92 million and 4.66 million in these 2 animals. At this time their red cell counts were 5.29

million and 4.07 million, respectively. After 24 hours, Monkey 62 had 5.49 million Heinz bodies and 5.30 million erythrocytes. The number of erythrocytes and Heinz bodies in both of these monkeys gradually decreased until the 5th experimental day, at which time Monkey 62 had 3.19 million red cells and 2.47 million Heinz bodies, while Monkey 63 had 3.57 million erythrocytes and 2.70 million Heinz bodies. The number of Heinz bodies progressively decreased until the 12th experimental day when none was demonstrated. The number of erythrocytes in the peripheral blood returned to normal by the 30th experimental day.

Heinz bodies in splenectomized monkeys given phenylhydrazine. Splenectomies were performed on 5 monkeys, 4 of which had been given phenylhydrazine hydrochloride previously. They were given phenylhydrazine for a second time and the number of Heinz bodies was observed. Monkey 63 (Fig. 2) was given 100 mg of phenylhydrazine hydrochloride after splenectomy. One hour later the Heinz body count was 3.16 million and 4 hours later the count was 4.63 million. On the 10th experimental day the number of Heinz bodies had decreased to 1.89 million and the erythrocyte count was 4.63 million. The number of red cells had returned to normal by the 21st experimental day: however, Heinz bodies persisted free within the peripheral blood until the 27th day. The Heinz bodies free in the plasma reached their maximum number on the 6th experimental day. Ten days after the Heinz bodies were last seen in the peripheral blood. Monkey 63 was given 20 mg of phenylhydrazine hydrochloride. Three hours later there were only 0.27 million Heinz bodies in the peripheral blood. They reached their maximum number, 0.45 M, on the 14th experimental day. The number of erythrocytes was not affected by this small amount of phenylhydrazine. Heinz bodies persisted in a few red cells for 84 days. Monkey 62 was treated the same as Monkey 63. The clinical course and the hematological changes were essentially the same in these 2 animals.

Monkeys 46 and 52 were given phenylhydrazine hydrochloride in small doses and the number of Heinz bodies free in the plasma was observed. Monkey 52 was given 50 mg of the drug each day for 3 days. Heinz bodies remained in the plasma of this animal until the 37th experimental day. Monkey 46 was given 100 mg of phenylhydrazine and 2 days later another 50 mg. Visible changes in the erythrocytes were present on the 3rd experimental day. Heinz bodies remained in the peripheral blood for 37 days.

Monkey 67, weight 2.6 kg, was given 80 mg of phenylhydrazine hydrochloride. One hour later the Heinz body count was 2.93 million (Fig. 3, Exp. 1). The count reached its maximum 24 hours later, at which time there were 4.41 million Heinz bodies and the erythrocyte count was 4.39 million. The number of Heinz bodies and erythrocytes gradually decreased until the 7th day when the red cell count was 1.67 million and the Heinz body count was 0.49 million. The Heinz bodies were not demonstrable after the 20th experimental day and the number of red cells had returned to normal by the 27th day. The number of Heinz bodies in the plasma per 500 erythrocytes during the time of this experiment is shown in Fig. 3. Eight days after completion of experiment 1 a 2nd dose of 80 mg of phenylhydrazine hydrochloride was given. The results are shown in Experiment 2 (Fig. 3). They are essentially the same as observed in the first experiment.

On the 6th experimental day Monkey 67 was very sick and unable to eat. He died on the 19th experimental day with extensive tuberculosis. No Heinz bodies were demonstrated on the day of death.

Discussion. The monkey has been used infrequently for the study of Heinz bodies(7), but it is obvious from these experiments that it is a very satisfactory host for such observations. Although there may be a wide variation in the response of different monkeys to the same quantity of the drug, the data in Fig. 3 indicate that this experiment may be repeated in the same animal with essentially the same results.

The changes produced within the red cell by phenylhydrazine are present within 30 minutes after a large dose is given. At this early time there is no change observed within the

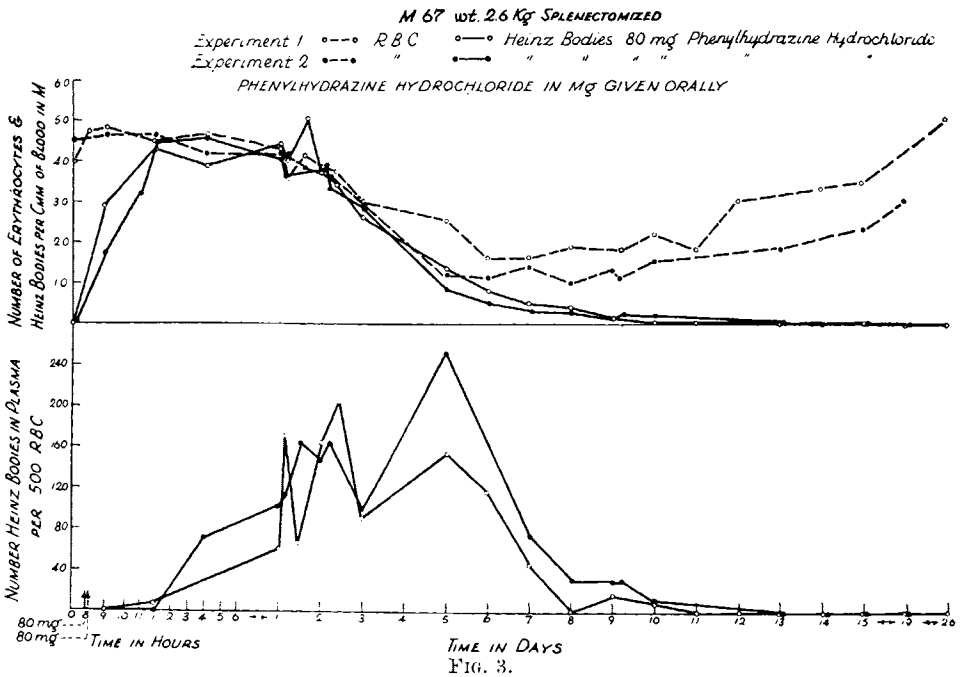

FIG. 3.

The experiment was repeated in this monkey. The results are essentially the same.

erythrocytes in routine blood smears stained with a combination of Wright's and Giemsa's stains. However, if at this time the red cells are put into distilled water, complete hemolysis does not occur. Subsequently, Heinz bodies become obvious within the erythrocytes and later these bodies become extracellular in blood smears. The change in the erythrocytes after a small dose of phenylhydrazine may be insufficient to result in free Heinz bodies in the blood smear (Fig. 1, Exp. 2). The number of Heinz bodies free in the plasma is proportional to the amount of the drug given. It should be noted, also, that the maximum number of Heinz bodies appears in the plasma following the time that the greatest number is present as shown by the direct counting method (Fig. 2 and 3). It is very difficult to count the Heinz bodies in blood smears when large amounts of the drug have been given since the cells and Heinz bodies are not distributed uniformly in the smear. Erythrocytes injured by phenylhydrazine hydrochloride are rapidly removed from the circulating blood of a normal monkey, while injured cells and Heinz bodies in splenectomized monkeys are removed at a much slower rate. Heinz bodies usually circulate in the peripheral blood of normal monkeys from 7 to 16 days and in splenectomized monkeys from 19 to 84 days. Cruz(3), using pyrodine, found that Heinz bodies remained in the peripheral blood of rabbits from 8 to 9 days and in dogs from 9 to 18 days. Webster and associates(7), using stibene, observed that these bodies persisted in the blood of guinea pigs for 11 days, in rats for 33 and in mice for 55 days.

The question arises of whether all erythrocytes injured by phenylhydrazine hydrochloride are removed from the peripheral blood. The resistance of some erythrocytes to the hemolytic action of distilled water would suggest that some of the cells, although affected, may continue to circulate for long periods. It is possible, also, that the change in the red cell is reversible when only a minimal change has been produced.

The method used in this study to determine the number of erythrocytes affected by phenylhydrazine hydrochloride is both simple and rapid. The ratio of Heinz bodies to the total erythrocyte count reflects the amount of phenylhydrazine hydrochloride absorbed.

In monkeys given a large amount of the drug, the number of cells affected is approximately the same as the total number of red cells. With smaller doses, the effect is proportionally less. Webster(1) comments on the fact that supravital preparations have "the advantage that the smaller Heinz bodies can be seen and thus the beginning stages of the phenomenon can be detected and followed." It should be remembered that a red cell affected by phenylhydrazine to such an extent that a Heinz body forms is also most likely to be affected to such a degree that either the cell or a portion of it will persist in distilled water and thus be counted in the total number of Heinz bodies. This technic could be used in routine clinical hematologic examinations and, if proven satisfactory, would be of value when drug or industrial poisonings are suspected.

Summary. Heinz bodies have been demonstrated in the blood of monkeys following the oral administration of phenylhydrazine hydrochloride. These bodies disappear from the circulating blood in normal monkeys more rapidly than they do from the blood of splenectomized animals. A quantitative method for determining the number of Heinz bodies has been described. This method is based upon the observation that Heinz bodies are not hemolysed in distilled water. A comparative study is made of the number of Heinz bodies free in the plasma in blood smears stained with a combination of Wright's and Giemsa's stains with those remaining after treatment with distilled water. The latter technic is both simple and rapid.

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Adenosine Triphosphate from Barium-Acid Insoluble Residues. (18450)

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The purpose of this paper is to demonstrate that the customarily discarded barium-acid insoluble residues obtained in the classical methods for the extraction of adenosine triphosphate may contain considerable amounts of a Ba ATP which differs markedly in solubility from ordinary ATP and to outline a procedure for its isolation. We first became interested in these residues following a relatively unsuccessful attempt to prepare ATP from whelk muscle by the method of Needham(1). The yield from the barium-acid soluble fraction of this material was disappointingly low while the acid insoluble residue was many times larger than the corresponding fraction obtainable from mammalian muscle. The procedure reported here, originally worked out for dealing with the whelk residue, has also been applied to the much smaller residues from rabbit skeletal muscle and beef heart with qualitatively similar results.

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Methods and Results. From approximately a bushel of whelks (Busycon sp?), obtained from a local fish market, 4.082 kg of foot muscle were removed. The muscle was homogenized in Waring Blendors in cold 10% trichloracetic acid, filtered through cheese cloth, and reextracted with an equal volume of cold 5% T.C.A. The filtrates were combined and centrifuged in a Sharples centrifuge. The residue was discarded and the supernatant fluid adjusted to pH 8.2 with concentrated NaOH. A precipitate formed which was centrifuged out and discarded. The cloudy supernatant was treated with 1 volume of cold ethyl alcohol; the precipitate which came down was removed by centrifugation and to the supernatant solution (12.6 liters) was added another 6.3 liters of alcohol; no additional precipitate was formed. The clear supernatant fluid (approximately 18.0 liters) was treated with 300 cc of 25% barium acetate, and placed in the cold room over night. The next morning the barium pre-