streptomycin as well as in media without streptomycin, while a second resistant strain grew only in a streptomycin concentration of 500 μg/ml with pyruvate and fumarate as carbon sources.

4. This second streptomycin resistant strain was transformed into a streptomycin-dependent strain under the above conditions. The dependent strain so obtained remained dependent after several transfers on nutrient agar containing streptomycin, and in turn gave rise to streptomycin-sensitive cells when a large inoculum was placed in streptomycin-free nutrient broth.

16796 P

A Simple Technic for Counting Megakaryocytes.*

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Principle of method. Make a thick drop preparation of an accurately measured volume of marrow as in examination for malaria and count all the megakaryocytes in the preparation.

Method. From a well-shaken, evenly suspended marrow specimen,[1] fill a Sahli hemoglobin pipette to the 20 cmm mark and blow contents onto a clean slide to form a thick drop. Spread evenly with the pipette tip over an oval area about 1.5 × 3 cm. Usually several such thick drop slides are made so that additional slides are available for examination in case part of the drop is detached by too vigorous washing and so that, when the count is low, a statistically significant number of cells can be counted.[1] Place slides in an incubator overnight. The following morning, immerse the slides in a solution of 5% formalin in 1% acetic acid in a Coplin jar until the erythrocytes are lysed and the thick drop is grayish in color. Flood the laking solution off the slide with water and gently rinse with buffer-phosphate solution (pH 6.4).[2] Stain with Wright's stain alone for 2 to 3 minutes. Add buffer-phosphate solution and stain 60 to 90 minutes. Wash slide by holding horizontally under gently running water. Air dry. Spread a thin film of immersion oil over the smear and examine systematically, using a mechanical stage and 200 × magnification, preferably with an 8 mm objective which is not immersed in the oil. Count all megakaryocytes seen in the specimen and multiply by 50 to express as number per ml. If the total nucleated marrow cell count per cmm and the leukocytic-erythrocytic ratio are determined, the number of megakaryocytes per million nucleated marrow cells or per million nucleated erythrocytic cells may be calculated. Morphology is fairly well preserved and any cell can be examined in greater detail by switching to the oil immersion lens.

In previously reported methods a count was made of all megakaryocytes and all nucleated marrow cells[3] or of all megakaryocytes and all nucleated erythrocytic cells[4] in an area of arbitrarily determined size on a thin marrow smear and results expressed, respectively, as megakaryocytes per million nucleated marrow cells or per million nucleated erythrocytic cells. These methods have the disadvantages that they are extremely time-consuming and that such large cells are seldom distributed evenly and, indeed, are likely to be found in greatest numbers at the tails of the smear. The area selected for examination will thus

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greatly influence the count. If results are expressed as number per million nucleated erythrocytic cells, megakaryocytes are actually more numerous than results would indicate in diseases characterized by erythrocytic hyperplasia such as blood loss anemia due to purpura hemorrhagica and actually less numerous than the results would indicate in erythrocytic hypoplasia. The same objections apply when the result is expressed as number per million nucleated marrow cells and, in addition, leukocytosis, leukemia, and other hyper- or hypoplastic diseases of the leukocytic system would distort the apparent number of megakaryocytes. For these reasons, it would be preferable to determine the absolute number per ml.

Study by this thick drop technic of the number of megakaryocytes found in normal and pathologic marrows is under way, but not yet complete. Preliminary results would seem to indicate that the normal range is between 500 and 4000 megakaryocytes per ml; between 20 and 150 per million nucleated marrow cells; and between 50 and 300 per million nucleated erythrocytic cells.

16797

Influence of Amino-Acids on Adrenal Enlargement, Nephrosclerosis and Hypertension by Anterior Pituitary Preparations.*

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Previous investigations carried out in this laboratory have shown that overdosage with lyophilized anterior pituitary (LAP) can produce nephrosclerosis and hypertension in rats fed a 30% casein diet.[1] It was also observed that different kinds of proteins[2] and protein hydrolysates[3] are as effective as casein in the production of nephrosclerosis and hypertension by LAP. This paper is concerned with the nephrosclerotic activity of LAP in animals fed a diet in which the protein component was provided by a mixture of crystalline amino-acids.

Material and methods. 25 hooded black-and-white male rats weighing 110 to 130 g were divided into 4 groups. Groups I and II received a "22%" amino-acid‡ diet and Groups III and IV a "22%" Amigen‡ (enzymatic casein hydrolysate) diet, both rations containing 4% sodium chloride. In order to maintain equal food intake, all animals received their diet as a 70 g % aqueous suspension, 3 times daily at 6-hour intervals through a stomach tube. First Groups I and II were adapted to this procedure by forced-feeding mixtures of Amigen and amino-acids in which the proportion of Amigen was gradually decreased so that on the 10th day only amino-acids were fed. At the same time, Groups III and IV were adapted to forced feeding with the Amigen diet. The volume and caloric value of the food given was the same in all groups. The amount of food was so adjusted that the animals slightly increased in weight.

After the adaptation period, all animals

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‡ "22%" means a nitrogen content corresponding to 22% casein. For composition of diets see Tables I and II.