other methods tried. The stain keeps indefinitely and need not be filtered before using. The oxalated blood may stand for as long as 48 hours before the count is made. Overstaining does not occur even though the smears are not made until 2 hours after the stain and blood are mixed. The reticulocytes are clearly and deeply stained and the red cells are neither crenated nor distorted. The smears keep indefinitely if counterstained with Wright's stain.

A reticulocyte stain on oxalated blood which had stood for 144 hours and was badly hemolyzed showed the same percentage of reticulocytes as did the fresh blood. This suggested to us that perhaps this is not a vital stain as is generally stated, but is merely a dye which will not stain fixed cells. To investigate this point further, equal parts of blood and 1% sodium cyanide solution were mixed and allowed to stand from 20 minutes to 1 hour and reticulocyte counts were done. These gave practically the same results as counts on the original blood. Cells fixed by various methods failed to show any reticulocytes.

Normal values for reticulocytes will have to be established by this method. Preliminary studies suggest that bloods of healthy adults will show about 2% reticulocytes.

5721

The Phosphatase Content of Fractured Bone.*

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In a recent paper Kay[1] states that he observed an increase in plasma phosphatase following fracture, and indirectly he suggests a similar rise in bone phosphatase. In view of our findings on the breaking strength of fractured fibulae of rats,[2] we were of the opinion that a positive correlation existed between the breaking strength and bone phosphatase. This we sought to demonstrate. It was also believed that if the activity of phosphatase in the healing process of fractures is antagonized, after the primary callus has formed on the fifteenth

*The expenses of this investigation were defrayed by Davis and Geck, Inc.

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[1] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

[2] McKeown, R. M., Lindsay, M. K., Harvey, S. C., and Lumsden, R. W., *Arch. Surg.*, in press.

day, by the hormone of the parathyroid gland, as we suggested elsewhere,[2] that subsequent to the fifteenth day the bone phosphatase should be reduced.

The method of selecting, feeding and fracturing the right fibulae of the albino rats has been described.[2] The bone phosphatase of the combined unfractured left fibula and tibia, as well as the bone phosphatase of the combined fractured right fibula and its tibia, were estimated by a method personally suggested by Kay,[1] the details of which will be published later. The results are tabulated in Table I.

TABLE I.
Average of Phosphatase Units Obtained.

Post-Operative Day	No. Animals	Right Fractured	Left Unfractured
2	2	17.7	15.6
4	2	13.3	13.9
6	3	19.1	18.0
9	3	22.8	24.0
12	2	16.5	15.1
15	4	19.2	19.8
18	2	13.9	13.5
21	2	9.3	9.6
24	3	16.8	15.3
27	2	8.2	9.3
30	2	13.1	11.0
33	2	7.0	6.9
36	2	7.2	5.9
39	2	16.8	14.6
42	2	8.5	9.1
45	2	11.0	10.2
	Controls (entirely free of fractures)		
0	6	12.8	13.6

Our findings, incomplete as they are, strongly indicate that the level of bone phosphatase in the fractured fibula rises with the formation of the primary callus and falls as the medullary space is developed. The rapid loss of callus strength during the formation of the medullary space, between the fifteenth and twenty-first days, has been suggested elsewhere as being due to a powerful circulatory decalcifying substance which may possibly be the parathyroid hormone.[2] The sharp reduction in the phosphatase content of the fractured right fibula and its normal tibia, subsequent to the formation of the primary callus on the fifteenth day, and during the formation of the medullary cavity, suggests that an actual antagonism does exist between phosphatase, and this at present unknown decalcifying substance.

The relatively close agreement between the phosphatase content of the unfractured left fibula and its normal tibia in the left leg, to

that observed in the fractured right fibula and its normal tibia in the right leg, offers additional proof for our previous reasoning, from the results of breaking strength determinations,[2] that the repair of a fracture is not as local as was formerly thought, and that the skeleton at large is called upon to furnish in a remarkably short space of time those substances essential for the healing of the fracture.

5722

Viability of the Cells of Rous Chicken Sarcoma Desiccate.

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It is a common experience in preparing tissue cultures, in transplantation and in supravital staining that desiccation is rapidly fatal to mammalian cells. It has usually been assumed that the tissues of all higher animals are similarly injured. Nakahara,[1] however, found in the desiccate of the Rous chicken sarcoma cells that he thought to be alive. He treated the cells of a desiccate more than 4 months old with dilute solutions of trypan blue and also inoculated fresh chicken plasma with them. By both methods cells thought to be living were seen.

Since Nakahara apparently observed the cultures for only a short time (3 or 4 hours) it seemed desirable to keep cultures under observation for a longer period. A preparation of desiccate was generously furnished me by Dr. Rous. It was prepared November 16, 1925, and on June 2, 1926, several dozen cultures were made employing the usual cover-glass method. The desiccate was first rubbed up with a small amount of Ringer solution to make a thick paste which was kept on ice until the explants were made. In one series whole fresh Barred Rock plasma was inoculated with the paste and in another the plasma was diluted with one-third volume of distilled water. The cultures were at once placed in the incubator and examined at 3 and 5 hours, one, two and five days. Round cells of medium size were seen on first examination and suggested growth. The nuclei were perfectly distinct and their irregular outline might be interpreted as evidence of ameboid motion. Many of these cells were located by ink marks on the cover-glass. After 5

[1] Nakahara, W., *Gann. Japanese J. Cancer Res.*, 1926, **20**, 13.