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Received January 3, 1956. P.S.E.B.M., 1956, v91.

Interaction of Gelatin-Stabilized Radiogold Colloid and Plasma Proteins. (22228)

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It has been demonstrated that the rate of disappearance of gelatin-stabilized radiogold colloid (Aurcoloid) from the circulation is inversely proportional to the amount of gelatin used to stabilize the Aurcoloid(1). Furthermore, the gelatin effect was enhanced by either injection of homologous rat plasma or alpha and beta globulin fractions of human plasma, whereas human serum albumin and gamma globulin had little effect. There was no evidence to suggest that the phagocytic capacity of the reticulo-endothelial system (RES) for the gold particles had been altered by gelatin. The present study was undertaken to investigate the interactions of gelatin with the plasma proteins as a possible explanation for this effect of gelatin.

Materials and methods. The Aurcoloid* was diluted with 50 volumes of distilled, demineralized water and stored in a refrigerator. The P-111 gelatin† solution, similar to that used by the manufacturer to stabilize the Aurcoloid, was handled under sterile conditions and maintained at 37°C in a water bath for at least 15 minutes before use. For the *in vitro* experiments, blood was obtained from adult male rats of the Wistar strain via the abdominal aorta using heparinized syringes. Human blood was obtained by venipuncture using heparinized syringes. After centrifugation, 3 ml volumes of plasma were placed in test tubes to which was added 0.05 ml of the diluted Aurocoloid and 2.5 mg of gelatin. Plasma samples were agitated for 2 minutes, incubated at 37°C for 30 minutes, then electrophoretically separated. In the *in vivo* experiments, adult male rats of Wistar strain weighing between 200-300 g were used. Animals were anaesthetized by intraperitoneal injection of nembutal, 5 mg/100 g of body weight. The external jugular veins were exposed and injections of the Aurcoloid

* Obtained from Abbott Laboratories, Oak Ridge, Tenn.

† Obtained from Charles B. Knox Gelatin Products, Camden, N. J.

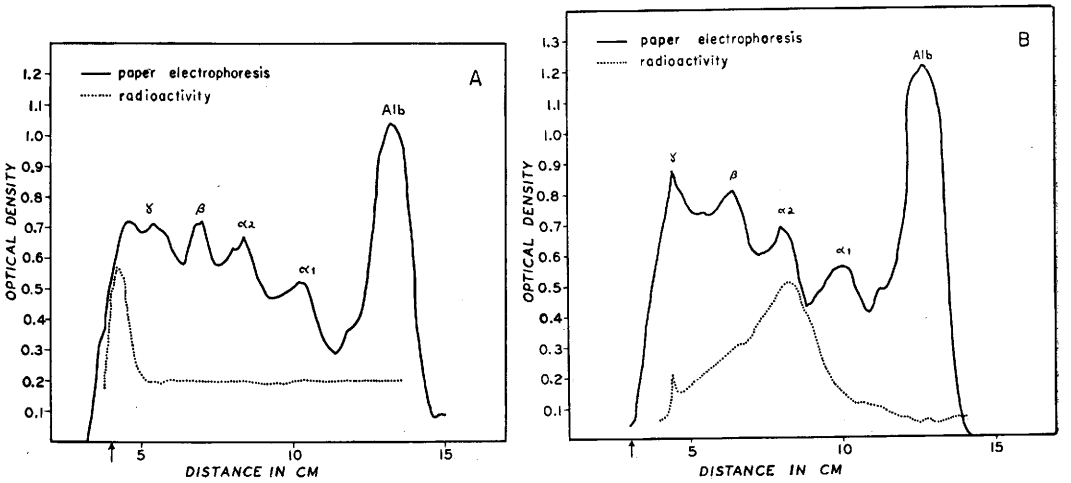

FIG. 1. Gelatin-plasma interaction *in vitro*. A. Human plasma plus radiogold. B. Human plasma plus gelatin and radiogold. Densitometric measurements of radioactivity made from radioautographs.

with and without added gelatin (2.5 mg/100 g of body weight) were given in one vein; blood samples (0.2 ml) were withdrawn from the opposite vein at various intervals using heparin coated syringes. Blood samples were centrifuged and the plasma was fractionated by paper electrophoresis. Paper electrophoresis was performed using a horizontal apparatus with the paper strips suspended by a central bridge. Four strips of Whatman 3 MM filter paper, 3.75 x 62 cm, were run simultaneously. Veronal buffer at pH 7.8 for rat plasma and pH 8.6 for human plasma and 0.05 ionic strength was used. After equilibration, 20 lambda of plasma was applied by streaking across a ruled line. A constant voltage of 100 volts was employed for 16 hours with a current of 4-5 milliamperes. After drying at 120°C for 30 minutes either radioautographs were made of the paper strips or the radioactivity on the strips was counted by a gas-flow chromatograph scanner driven by a synchronous mechanical feed from an Esterline-Augus recording apparatus. Thus the radioactivity patterns could be directly superimposed on the plasma protein patterns. The paper strips were then stained with bromphenol blue. Densitometry of the paper strips and radioautographs was performed with a Photovolt Model 501A instrument with optical density recorded at 2 mm intervals.

Results. When Aurcoloid alone was added to human plasma *in vitro,* the radioactive material did not migrate but remained at the origin. However, if gelatin and Aurcoloid were added to plasma the radiogold now had a mobility which corresponded with the alpha 2 globulin fraction (Fig. 1). Similar experiments using rat plasma gave the same results.

In the *in vivo* experiments the effect of gelatin was similar to that seen in the *in vitro* studies. Paper electrophoresis of plasma obtained from animals previously injected with gelatin and Aurcoloid demonstrated the similar location of the radioactive material and the alpha globulin fraction, whereas the radioactive gold did not migrate when Aurcoloid alone was injected (Fig. 2). The rate of disappearance of the Aurcoloid from the blood was markedly retarded in the gelatin injected animals (compare A and B, Fig. 2).

The relative mobilities of gelatin and the alpha globulin fractions of rat plasma were demonstrated by paper electrophoresis. Samples containing various combinations of Aurcoloid, gelatin and plasma were placed on filter paper and run simultaneously in the electrophoresis chamber. Aurcoloid alone, or in plasma, did not migrate. When Aurcoloid was added to gelatin the radiogold migrated with the gelatin whereas the combination of Aurcoloid, gelatin and plasma resulted in a

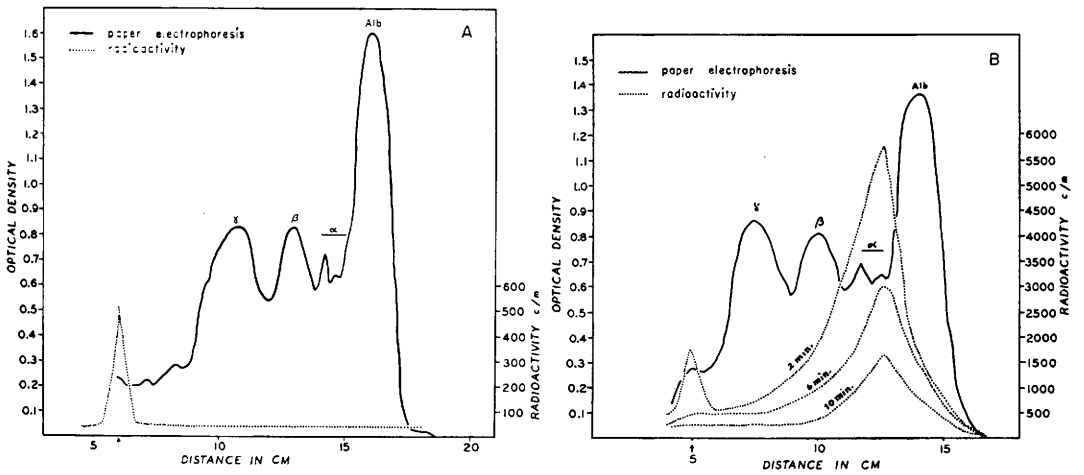

FIG. 2. Gelatin-plasma interaction *in vivo*. A. Blood sample removed 2 min. after radiogold injection. B. Blood samples removed at intervals after radiogold plus gelatin injection. Total radioactivity injected was similar in A and B.

still greater mobility of the radiogold which corresponded with the alpha globulin fraction (Fig. 3). These experiments suggested interaction between gelatin and the alpha globulin fraction and the possibility that radiogold particles are merely incorporated in the gelatin mass. The formation of a radiogold-gelatin-alpha globulin complex was associated with retention of radiogold in the circulation for longer intervals than was the case when interaction could not be demonstrated.

Discussion. Binding of radiogold colloid to the alpha and beta globulin fractions of human plasma, as demonstrated by Simon (2), may be explained by the fact that commercial radiogold preparations are stabilized by gelatin. The evidence presented in this investigation suggests that gelatin rather than

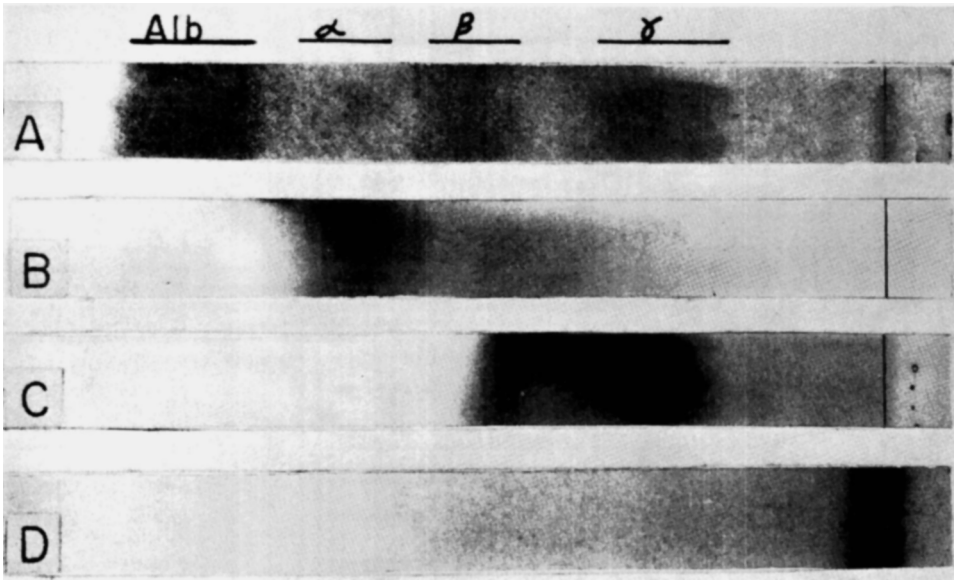

FIG. 3. Comparative electrophoretic mobilities of radiogold colloid. A. Electrophoretic pattern of rat plasma. B. Autograph: radiogold plus gelatin in plasma. C. Autograph: radiogold in gelatin. D. Autograph: radiogold in plasma.

metallic gold was bound by the alpha globulins. Although radiogold salts have been shown(3) to interact with the albumin fraction of plasma, Aurcoloid is relatively free of ionic gold.

The effect of gelatin in prolonging the retention of Aurcoloid in the circulation was apparently not mediated at the cellular level since colloidal gold was rapidly ingested by the RES regardless of whether the gold particles were coated with gelatin or plasma proteins(4). As Aurcoloid was eventually removed from the circulation by the RES, either the gold-gelatin-alpha globulin interaction was reversible or the total complex was phagocytised. Dissociation of the complex was not demonstrated in plasma electrophoretic patterns of animals injected with gelatin and Aurcoloid.

Summary. The relative mobilities of Aurcoloid and gelatin have been demonstrated *in vivo* and *in vitro* by paper electrophoretic methods. Addition of Aurcoloid and gelatin to plasma resulted in a radiogold-gelatin complex which migrated at the same rate as alpha globulins. Aurcoloid in plasma did not migrate. The formation of an Aurcoloid-gelatin complex was associated with a decreased rate of disappearance of radiogold from the circulation.

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Received January 4, 1956. P.S.E.B.M., 1956, v91.

Action of Parathyroid Extracts on Stable Bone Mineral Using Radiocalcium as Tracer.* (22229)

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When radiocalcium is injected into rats it becomes deposited in the skeletons of the animals in 2 forms which differ greatly in relative ease of mobilization and excretion. These forms of incorporation of radiocalcium and the parts of the skeletal bone mineral associated with each are referred to as the stable bone mineral and labile bone mineral(1,2). Fifty-two days after injection, radiocalcium is distributed almost exclusively in the stable fraction.

The present work describes the action of subcutaneously injected parathyroid extracts on radiocalcium (Ca^{45}) incorporated in the stable bone mineral.

* This investigation was supported in part by a grant from U. S. Public Health Service.

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Procedure. Twenty-four adult-male rats of comparable age and weight (380-420 g) were given intraperitoneal injections of high specific activity radiocalcium. This was administered in physiological saline solution in 3 doses of 2 cc each, allowing 2 hour intervals between injections. The total injected dose amounted to approximately 9.9 x 10^5 counts per minute according to the methods of sample preparation and counting conditions employed. After 72 days the left foreleg of each rat was amputated at the shoulder. Within this time the radiocalcium had presumably been distributed preponderantly in the stable fraction of the skeleton(1,2). The humeri of the amputated legs were divested of adhering flesh and stored in a freezer to be compared with opposite pairs from the same animal obtained later at the time of sacrifice. One day after removal of the legs the rats were given a subcutaneous injection of 100 USP units of