When purified nitrogen was bubbled through cultures of the basic medium irregularities were absent for the first seven hours of incubation (lower portion of Fig. 1).

Thus it would appear that the potentials that have ordinarily been attributed to the production of measurable oxidation-reduction systems by growing organisms are to a certain extent dependent upon the presence of poising materials present in the medium before growth is initiated. Such a poising agent can act as a mediator between the bacteria and their systems and the electrodes. Preliminary experiments have supported this concept. Stratification of growth has been shown to occur in some media and it seems that the more comparable results obtained by bubbling nitrogen or other gases through a medium might in part be due to agitation in addition to the removal of oxygen electrode effects. Simple mechanical agitation introduces complications because of the oxygen lability of the systems present.

9889 P

Antisera for Organ-Proteins.

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In none of the experimental work conducted in this laboratory could tissue-proteins be completely freed from blood proteins, nor has it been found possible to separate the blood proteins quantitatively from the autolyzed tissue-protein. So, antisera to tissue-protein or to autolysates always contained precipitins for blood proteins. Absorption *in vitro* with blood proteins usually removed not only their precipitins but also any precipitins that may have been formed for tissue-proteins. One investigator, Gilman,[1] working with urinary protein from a patient with severe chronic nephritis, was able to remove quantitatively the precipitins for hemoglobin, pseudoglobulin, and albumin by absorption *in vitro,* and obtained a precipitin that was specific for renal protein as found in an autolysate of the kidney When the renal autolysate was used for immunization, absorption *in vitro* of the blood-protein precipitins removed all of the precipitins.

[1] Gilman, G., *J. Urology*, 1935, **34**, 727.

Gay and Rusk[2] " . . . obtained some evidence that the critical reinjection of horse serum causes a temporary fall in precipitin content." Hektoen and Welker,[3] working with pure antigens, were able to remove certain specific precipitins by intravenous injection of corresponding antigens without affecting other precipitins present in the immunized rabbit.

Fresh human organ-tissue was ground in a meat chopper, washed several times in saline in an attempt to wash out as much of the blood proteins as possible, and then ground for 48 hours in a ballmill with saline and toluene. The tissue was again washed in saline in the centrifuge until freed from practically all water-soluble protein. The finely divided tissue was strained, fixed on aluminium cream, and injected into the leg and thigh muscles of rabbits—about 3 cc per depot until 30 cc had been injected into each animal.

In 10 to 14 days precipitins for hemoglobin, pseudoglobulin, and albumin were found in high titer. Equal parts of 1% human hemoglobin, 1% human pseudoglobulin, and 1% human albumin to make 10 cc were then injected into the marginal ear vein of these animals. In 8 hours the precipitins for albumin had disappeared; in 10 hours the precipitins for hemoglobin and pseudoglobulin were also gone. Blood was drawn from the rabbit at 6-hour intervals and the serum tested for the above precipitins. In 36 hours after the injection the precipitin for hemoglobin had reappeared and in 42 hours those for pseudoglobulin and albumin were present. Serum collected earlier, when free from blood-protein precipitins, gave positive precipitative reactions with hepatic autolysates.

From rabbits injected with ground liver and kidney, the precipitins for blood proteins disappeared after 4 to 8 weeks, but the serum still reacted with autolyzed kidney and liver. This was not true in all the rabbits injected. In some, the interval was considerably longer and in one the precipitins for the organ-proteins disappeared with the precipitins for the blood proteins.

Fresh extracts, prepared by grinding the tissues in a mortar with sand and saline, were not specifically precipitable until the tissues had autolyzed. Autolyzing (37°C) liver tested at one-hour intervals for 4 hours and at 4-hour intervals for the next 48 hours, gave positive reactions, except the autolysate at the end of the first hour. Kidney-extract tested at 6, 12, 18, 24, 48-hour intervals gave positive reactions with kidney antiserum. Both systems cross-reacted. The liver antiserum precipitated with autolysates of adrenal, pan-

2 Gay and Rusk, *Univ. California Publ. Path.*, 1912, **2**, 59.

3 Hektoen, L., and Welker, W. H., *J. Inf. Dis.*, 1935, **57**, 337.

creatic, splenic, and thyroid tissue; brain-tissue was negative. Kidney-antiserum, tested with these autolysates, gave negative or unsatisfactory results. Only very low dilutions of the renal autolysates were reactive but precipitation occurred in high dilutions (1-100,000) of the hepatic autolysate.

Summary. Precipitins for blood proteins disappeared from the circulating blood of rabbits immunized with renal and hepatic tissue as soon as these antigens are exhausted from the injected-tissue depots. The exhaustion of the tissue-proteins usually takes a much longer time. This provides a method for the preparation of tissue-antisera. Extracts of fresh liver and kidney do not give precipitative reactions with the antisera, but the autolyzed products of these organs do. Liver-autolysate seems to have an antigenic factor in common with the autolysate of kidney, pancreas, spleen, adrenals, and thyroids. Kidney-autolysate has an antigenic factor in common with liver but not with any of the other above-mentioned organ-autolysates.

9890

Effect of Inorganic Phosphorus on Bone Growth and Repair.

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That the feeding of inorganic phosphorus will produce transverse lines or bands of increased density in the metaphyses of growing long bones has been demonstrated by means of roentgenograms by Phemister[1] and subsequently by various observers. This "phosphorus band" has been thought to be a "growth arrest line," but the hypothesis has not been proved. Such lines have been observed only where longitudinal growth has taken place. They are not produced by supplements of Vitamin D without phosphorus.

We observed in the roentgenograms of a few young patients who were receiving phosphorized cod liver oil, dense new bone about areas of healing fractures or osteotomies of long bones. The callus about the osteotomy site, along the edges of the chisel cut, appeared to show an increase in density similar to the phosphorus lines in the metaphyses of the same bones.

[1] Phemister, D. B., *J. A. M. A.*, 1918, **70**, 1737.