and SH-compounds. (3) The compounds may react with acetaldehyde, the chief reaction product; thus removing its influence. Lohman and Schuster[4] have shown that acetaldehyde inhibits the system and Schubert has observed compound formation between SH compounds and the former. The first possibility appears to us to be the most likely.

The work is being continued and will be reported in more detail at some future date. On the basis of the present work, the assay of tissues and biological fluids for cocarboxylase by the method of Ochoa and Peters[2] or Goodhart and Sinclair[5] may be open to question, since most tissues are rich in SH-compounds. This may offer an explanation for the results obtained by Lipschitz, *et al.*,[6] who found that livers of polyneuritic chicks sometimes gave values for cocarboxylase which were higher than those found for normal birds.

11243

Toxic Effect of Human Urine on Fibroblasts Growing *in vitro*.

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It has long been held that urine possesses toxic properties. Bouchard[1] demonstrated the toxicity of urine by injecting it into the ear vein of a rabbit, determined how much was needed to kill the animal, and called quantity required per kg of body weight the urotoxic coefficient. This method came to be employed for determining the toxicity of the urine in various physiological and pathological conditions. Diminished toxicity in certain pathological conditions, especially uremia, was ascribed to the retention of toxic substances in the blood and was held to be the cause of some or all of

3 Schubert, M. P., *J. Biol. Chem.*, 1936, **114**, 341.

4 Lohman, K., and Schuster, P., *Biochem. Z.*, 1937, **294**, 188.

5 Goodhart, R. S., and Sinclair, H. M., *Biochem. J.*, 1939, **33**, 1099.

6 Lipschitz, M. A., Potter, V. R., and Elvehjem, C. A., *Biochem. J.*, 1938, **32**, 474.

* With the assistance of Miss E. Nussbaum.

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1 Bouchard, quoted by Volhard, *Bergmann und Staehelin's Handbuch der Inneren Medizin*, Berlin, 1931, **6**, 720.

the clinical symptoms. These experiments were the general basis for the conception of auto-intoxication.

The toxicity of the urine was later clearly demonstrated by Bruecke[2] who produced severe uremic symptoms in dogs by anastomosing the ureter of one kidney with the common iliac vein, the other kidney remaining intact. This was repeated and confirmed by Hartwich and Hessel[3] and Enderlen, Zuckschwerdt and Feucht.[4] These experiments led to the conclusion that the toxic effect of urine is not due to constituents preformed in the blood, but that the urine must contain toxins formed in the kidney.

More recently Rohdenburg and Nagy[5] have demonstrated the presence in normal human urine of growth-inhibiting as well as of growth-promoting substances, using the rate of division of the protozoon *Colpidium campylum* as an index.

A report is here given of the toxic effect of normal human urine on fibroblasts growing *in vitro*. The experiments were carried out in connection with an investigation into the toxicity of uremic blood and urine, which will be reported elsewhere.

Heart fragments of uniform size from 7-day-old chick embryos were used for explantation. After 3 passages in hanging drops they were transformed to flasks and cultivated according to the standard method of Carrel. Chick's plasma diluted with Tyrode in the proportion 1:2 coagulated by one drop of diluted embryonic extract constituted the solid phase of the media.

Normal human urine (sp. gravity 1025-1030) was taken from various healthy individuals and added in varying concentrations to the flasks; the diluent used was Tyrode or embryonic extract. The urine was taken sterile or sterilized by filtering through a Jena glass filter (5/3). The pH of the supernatant fluid of the experimental flasks with concentrated urine (0.5 cc urine diluted with the same amount of Tyrode) was practically the same as that of the control flasks; it varied from 7.6 to 7.9.

In order to establish that the hypertonicity of the concentrated urine does not interfere with growth the effect on growth rate of fibroblasts of a sodium-chloride solution of the same specific gravity as the urine (1026) was examined and the experiment showed that the cultures grow in this salt solution (in amounts equivalent to the urine) just as well as in the control flasks with Tyrode.

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2 Bruecke, E. T., *Wien. Klin. Wschr.*, 1926, **38**, 1058.

3 Hartwich, A., and Hessel, G., *Klin. Wschr.*, 1927, **35**, 1650.

4 Enderlen, Zuckschwerdt and Feucht, *Mchner. Med. Wschr.*, 1928, **75**, 30.

5 Rohdenburg, G. L., and Nagy, S. M., *Am. J. Canc.*, 1937, **29**, 66.

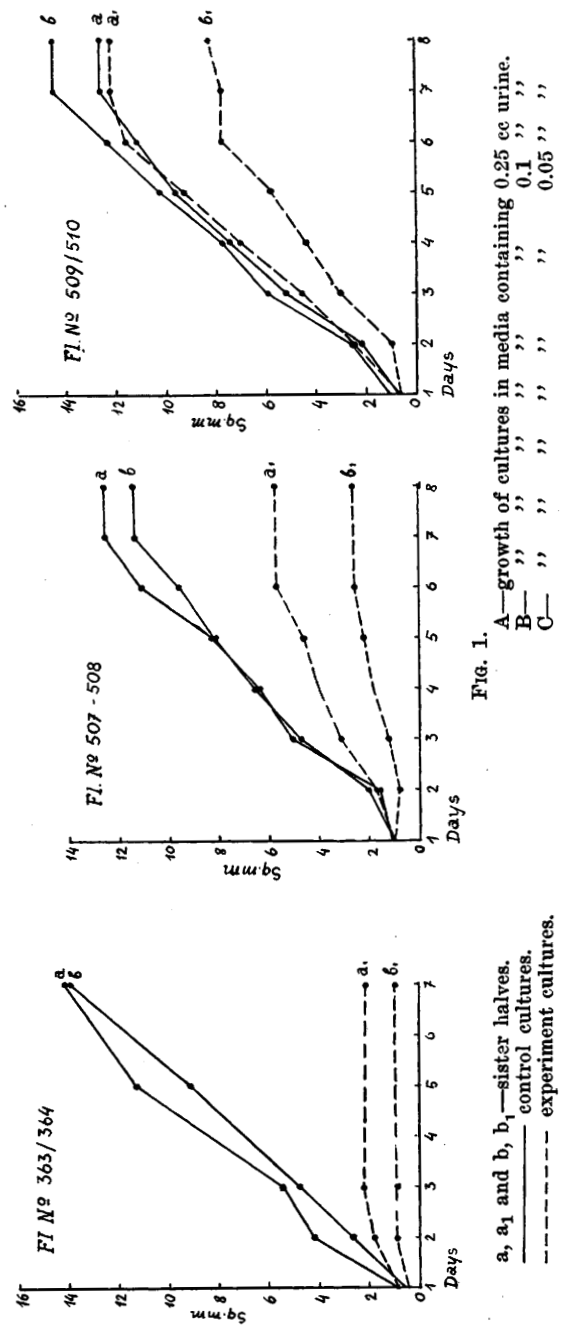

FIG. 1.

A—growth of cultures in media containing 0.25 cc urine.
B— " " " " " " 0.1 " "
C— " " " " " " 0.05 " "

The experiments were made with sister halves of cultures, one half serving for the test, the other half for control. The cultures were observed for 5-8 days, during which period the medium was not changed. They were projected every day and drawn and measured with a planimeter in accordance with Ebelling's method. The resulting values were used in plotting the growth curves.

The experiments showed that normal human urine causes an inhibition of growth as well as degenerative changes in the fibroblasts.

The addition of 0.5 cc urine (bringing the ratio of urine to flask contents to 1:5) completely inhibits growth in the tissue fragments *in vitro*. When 0.25 cc urine (final dilution 1:10) was added, a just perceptible growth was observed (Fig. 1A). After the addition of 0.1 cc (final dilution 1:25) there was marked growth but distinctly less than in the controls (Fig. 1B). An inhibition of growth was also generally noticeable with 0.05 cc urine (end dilution 1:50), (Fig. 1C). With higher dilution of the urine up to 0.025 cc (end dilution 1:100) no difference was observed in the growth rate of the test-cultures as compared with the controls.

The colonies growing in media containing urine show not only inhibition of growth but also considerable degenerative changes in the cells. The growing zone appears dark, dense, the cells markedly granular, frequently loosely connected.

In cultures growing in media containing urine and embryonic extract, the effect of the urine was practically the same as in cultures without embryonic extract.

A further series of experiments was undertaken with a view to investigating the nature of the urinary toxic substances. So far it

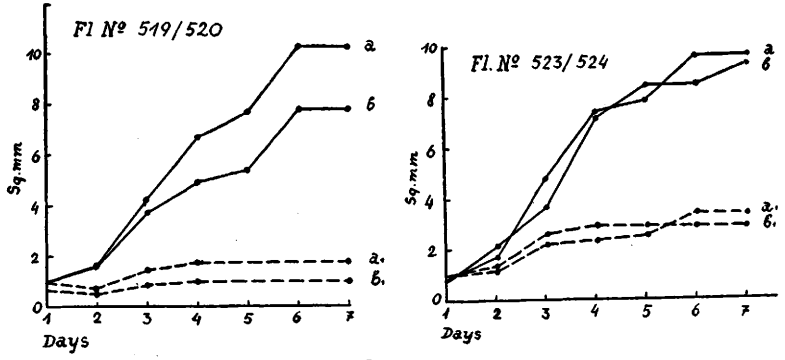

FIG. 2.

a, a_1 and b, b_1—sister halves.
———— control cultures.
– – – – – – – – experiment cultures.
A—growth of cultures in media containing 1 cc of the residue after dialysis.
B— " " " " " " 0.5 cc of the residue after dialysis.

has been shown that the toxicity of urine for fibroblasts is not destroyed by heat. Urine heated to 60°C and 100°C causes the same degree of inhibition of growth as unheated urine. It was also shown that the toxic substance in urine is non-dialysable. The addition to cultures of 1 cc or 0.5 cc of the residue after dialysis (corresponding more or less to the same volume of urine) results in a markel inhibition of growth (Fig. 2A and B), though somewhat less than is seen with whole urine.

The method described here of demonstrating the toxicity of urine by its effect on the growth rate of fibroblast colonies is thus exceedingly sensitive and enables us to measure the toxicity of urine with a great degree of exactitude. This method may therefore help in the investigation of the nature of the toxic substance present in urine as well as the toxicity of urine in various pathological conditions.

Summary. A method is described of demonstrating the toxicity of urine by its effect on the growth rate of cell colonies *in vitro*. Normal human urine in a dilution 1:5 completely inhibits the growth of fibroblasts; urine in a dilution of 1:50 still perceptibly inhibits their growth. The cells growing in media containing urine even in small concentrations exhibit signs of degeneration. Heat does not destroy this property of urine. The toxic substance is nondialysable.

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Effect of Concomitant Administration of Estrogens and Progesterone on Vaginal Smear in Man.*

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Of the changes in the vaginal secretion during the menstrual cycle[1] those occurring in the first half concurrent with the growth and ripening of the ovarian follicle are most clearly defined and best understood. The demonstration in this laboratory[2] that they are similar to the smear changes induced in menopause and amenorrhea

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