during period when aspirin in solution was given ($F = 0.57$, $P > 0.10$).

*Discussion.* Previous investigators(2-4), using qualitative tests for occult blood in feces concluded that gastrointestinal bleeding induced by aspirin occurred: a) with both soluble and insoluble aspirin, b) in subjects with or without demonstrable gastrointestinal lesions, and c) in subjects with or without history of hemorrhage while ingesting aspirin. These findings are substantiated by the present study with quantitative measurements of fecal blood loss.

In no instance could bleeding under these experimental conditions be classified as clinically important gastrointestinal hemorrhage. The factors involved in patients who experience major hemorrhage while ingesting aspirin await elucidation.

*Summary.* In humans whose erythrocytes were tagged with $Cr^{51}$, fecal excretion of the isotope was used to measure gastrointestinal blood loss. In 38 subjects, ingestion of 3 g of aspirin/day for 3 or 6 days increased fecal blood loss of 3.3 ml/day. Patients who had recently had upper gastrointestinal bleeding and patients who gave a history of gastrointestinal hemorrhage while taking salicylates did not differ in their response from subjects without such histories. Blood loss was smaller in subjects who received antacids every waking hour while taking aspirin than in those who did not receive antacids. Alkaline solution of aspirin produced the same effect as aspirin tablets.

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## Photosensitization of Tissue Culture Cells and Its Effect on Viral Plaque Formation.* (25303)

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The phenomenon of photosensitization or photodynamic action of dyes and other chemical compounds is well known, and has been observed to affect various microorganisms and cells, as well as plants, animals and man(1). Stilwell described the photodynamic action of neutral red on cultures of embryonic chick cells(2), but no report has been found of photosensitization of agar-overlaid tissue cultures by the neutral red which is commonly contained in the overlay medium. On the other hand, neutral red itself has been reported to be lethal for some cell cultures, and to reduce viral plaque counts in others(3). During the course of experiments involving the use of rhesus monkey kidney culture cells for the study of viral plaques, prepared as originally described by Dulbecco(4) and modified by Hsiung and Melnick(5), it was noted that occasionally entire cell sheets or portions of sheets in some bottles inexplicably deteriorated. This deterioration was evidenced by a change in color from the usual pinkish-red to orange or yellow, and a homogeneous appearance of the cell sheet and agar overlay, similar to that seen in the confluent degeneration caused by infection with certain viruses, and generally interpreted as being indicative of death of the cells. Cultures exposed to direct light during incubation in a lighted "walk-in" incubator usually were the ones affected.

*Methods.* The hypothesis that photosensitization caused such destruction was studied by exposing cultures to a standardized source of light. A commercial bacteriological incu-

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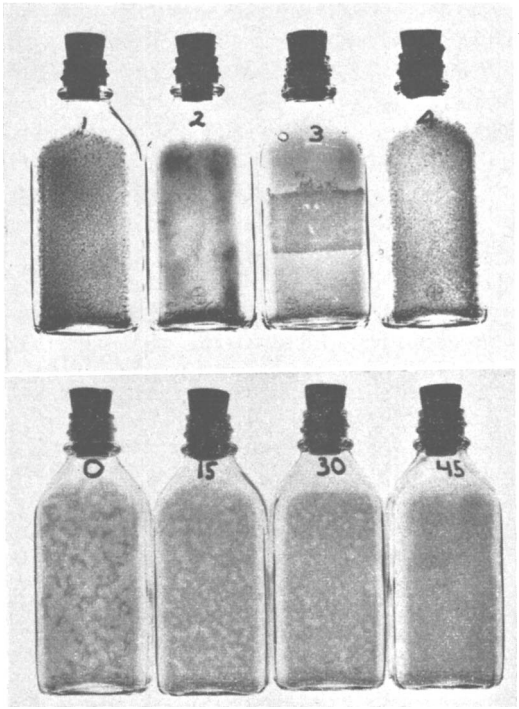


FIG. 1 (top). Effect of exposure to light on cultures of rhesus monkey kidney cells. Bottle 1, not exposed to light, shows intact sheet of cells; in bottle 2, exposed to light in presence of neutral red, no cell sheet is visible; bottle 3, treated in same manner as #2 except for application of a strip of masking tape during period of light exposure, shows only a band of the cell sheet remaining in area shaded from light; bottle 4, exposed to light in absence of neutral red shows a good cell sheet indistinguishable from that of bottle 1.

FIG. 2 (bottom). Effect of exposure to light on plaque formation of type 1 poliovirus in rhesus monkey kidney cells. Photograph was taken 48 hr after each culture had been inoculated with 100 plaque forming units of virus. Bottle 0 was not exposed to light; the others were exposed to light 15, 30, and 45 min, as designated. Note apparent reduction in size and No. of plaques which decrease progressively with increases in time of exposure.

bator, measuring 20 × 22 × 30 inches on the inside, was equipped with a fluorescent light fixture consisting of two, 15-watt, white units, attached to the ceiling. Bottles containing cultures were exposed to direct light by placing them in a tray on a shelf 20 inches beneath the light source. One half of the tray was covered by a piece of plywood, and cultures placed in the covered half of the tray were thus protected from exposure to direct light and served as controls. Most experiments employed rhesus monkey kidney cells grown in 3-ounce prescription bottles, and overlaid with agar containing neutral red in a final concentration of 1:60,000, in accordance with a commonly employed procedure of plaque assay(5). In order to determine that the degenerative changes observed were due to the photodynamic effects of neutral red, some cultures were overlaid before exposure to light in exactly the same manner except that the overlay medium contained no neutral red. A typical experiment consisted of preparing 8 bottle cultures, 4 with an overlay medium containing neutral red and 4 with the same medium without neutral red. As soon as the agar overlay had solidified, 4 bottles—2 with and 2 without neutral red—were exposed to light, while 4 similar bottles were placed in the same tray but shaded from light, in the manner described above. After exposure, cultures without neutral red in the first overlay received a second overlay containing neutral red, whereas those with neutral red in the first overlay received a second overlay without neutral red. All cultures were then incubated in the dark for a day or two.

*Results.* The cell sheets of cultures which had not been exposed to light had the usual fine lacy, pinkish-red appearance; moreover, those which had been exposed to light in the absence of neutral red presented a similar appearance, while the cell sheets of cultures exposed to light in the presence of neutral red actually could not be recognized, and the contents of bottles containing such cultures appeared homogenous and pinkish-yellow or orange. Exposure for as little as 2 or 3 hours resulted in visible degenerative changes. Furthermore, cultures which had developed the typical pinkish-red appearance after application of the usual overlay containing neutral red and incubation in the dark, when subsequently exposed to light, underwent similar deterioration. The photosensitizing action of neutral red was demonstrable with both fluorescent and incandescent light; and tissue cultures of Hep 2, chick embryo kidney, and dog kidney, also were found to be susceptible. Fig. 1 demonstrates the effects of exposure to light on photosensitized monkey kidney cells.

The possible influence of photosensitivity on viral plaque formation was also studied.

Titrations, in quadruplicate, of type 1 poliovirus (Mahoney strain) were carried out, by the plaque-assay technic, in bottle cultures of rhesus monkey kidney cells. Cultures inoculated with various amounts of virus were exposed to light for varying periods of time, and compared with control cultures which had not been exposed to light. Periods of exposure insufficient to produce discernible changes in the cell sheets were chosen. After 48 hours of incubation in the dark, following infection, plaques were often found to be smaller and less numerous in the cultures exposed to light than in unexposed controls. On further incubation, however, these differences were minimized or obviated. Fig. 2 illustrates the effect of light on the plaque formation of type 1 poliovirus in tissue cultures.

*Summary.* Degenerative changes occur in agar-overlaid tissue culture cells which have been exposed to light in the presence of neutral red. Temporary modification of the ability to form poliovirus plaques may occur in such cells when exposure to light is insufficient to produce discernible changes in the cell sheet.

*Addendum.* After this paper had been submitted for publication a report (Klein, S. W., Goodgal, S. H., *Science,* 1959, v130, 629) appeared which contained data similar to that presented above.

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## Action of Testosterone in Blocking Urinary Electrolyte Effects of Desoxycorticosterone.* (25304)

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Two distinct types of steroids were reported recently to antagonize renal electrolyte effects of mineralocorticoids. The steroidal 17-spirolactones(1) were discussed as first synthetic examples with aldosterone- and desoxycorticosterone-blocking activity in the laboratory (2-4) and in man(4,5). By virtue of electrolyte effects which were dependent upon 1) presence of mineralocorticoids and 2) dosage ratio of blocking agents/mineralocorticoids, the mechanism of action of spirolactones was ascribed to competitive inhibition. A naturally occurring steroid, progesterone, was also reported to possess a similar blocking ability(6-9), but it demonstrated desoxycorticosterone-like effects on electrolyte excretion when given alone at large doses(6). The present report summarizes ability of testosterone, another gonadal hormone, to reverse electrolyte effects of desoxycorticosterone in rats.

*Method.* Sprague-Dawley male rats (150 to 200 g) were adrenalectomized under ether anesthesia, then maintained on 0.85% sodium chloride solution and Purina Chow *ad lib.* Approximately 6 days postoperatively animals were injected subcutaneously with 2.5 ml isotonic saline and with 12 $\mu$g of desoxycorticosterone acetate (DCA) in 0.1 ml Mazola oil. Groups were injected additionally with 0, 2, 4, 8 and 20 mg of testosterone in 0.5 ml for study of blocking effects. Other animals received corresponding doses of testosterone without DCA. Animals were placed in metabolism cages (5 rats/cage) for collection of 4-hour sample of urine. Pooled urine from each cage was diluted with water and analyzed for total Na and K content using Beckman flame photometer. Values for each sample were used directly for computation of the Na/K ratio. DCA alone reduces the ratio of

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