TABLE 1

EFFECT OF *Tetrahymena geleii* ON INFLUENZA B VIRUS

Protozoal culture*	Viral hemagglutinin titer			Infective titer (ID_{50})
	Hr incubation at 28° C			
	0	24	48	48
None	64†	32	32	$10^{-6.5}$
Viable				
Undiluted	32	32	64	$10^{-2.7}$
½‡	32	32	16	
1/20	32	32	1	$10^{-4.3}$
Killed (frozen and thawed):				
Undiluted	32	32	32	$< 10^{-1.0}$
½	32	32	32	
1/20	32	32	32	$10^{-4.6}$

* Previously incubated 7–9 days at 28° C.
† Reciprocal of hemagglutinin titer of culture.
‡ Diluent used was 1% proteose peptone medium.

groups of 5 eggs each. Macroscopic and microscopic examination of the cultures at various intervals indicated that the presence of virus did not affect either the motility of the protozoa or their rate of multiplication. Little or no gross change in the concentration of protozoa was observed in mature undiluted cultures in the presence or absence of virus. However, the virus was affected in several ways by the protozoal culture.

The data from a typical experiment are summarized in Table 1. When the infective titers of the various cultures are compared after 48 hr of incubation it is evident that both viable and killed cultures of *T. geleii* contained a factor which inactivated influenza B virus. Protozoal cultures, killed by freezing and thawing, were found to be slightly more effective than viable cultures. Dilution of the protozoal cultures was accompanied by diminished viral inactivation. These data recall to mind the fact that antibacterial lipids have been obtained from similar cultures of *T. geleii* (*3*). Further inspection of the data indicates the existence of another phenomenon. It will be seen that viral hemagglutinin was markedly reduced by diluted, actively multiplying, viable protozoal cultures, whereas killed cultures or undiluted, mature, viable cultures had no effect on viral hemagglutinin. Identical end points (1/320) were obtained when viral hemagglutinin was titrated in the presence of culture medium from young (3-day) protozoal cultures, fresh broth, and saline, respectively. It is clear, therefore, that viral hemagglutinin, representing both infective and noninfective virus particles, was inactivated or destroyed only by actively multiplying (i.e., diluted) protozoal cultures. Similar results were obtained in comparable experiments with influenza A virus. It is well known that *T. geleii* and other ciliates feed upon certain bacteria and it would appear that a similar mechanism obtains in the case of the influenza viruses.

The influenza viruses, then, do not affect *T. geleii*, but the protozoal culture may affect the virus in at least 2 ways: first, viral inactivation by a factor present in both killed and viable cultures, and, second, inactivation or destruction of viral hemagglutinin by actively multiplying but not by mature protozoal cultures.

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The Importance of Protective Urinary Colloids in the Prevention and Treatment of Kidney Stones

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A calculus is generally defined as a concentration formed of crystalloids (*1*) held together by, and incorporated in, a colloidal matrix. The formation of urinary calculi is, however, far more complex and is still not fully understood (*2–6*).

Most investigations concerning the prevention and treatment of kidney stones have so far been directed only toward trying to ascertain how the concentration of crystalloids excreted in the urine could be diminished. With the exception of correcting certain metabolic disorders, all attempts to control calculous formations have so far not proved entirely successful. The influence that hydrophilic colloids exert in the etiology of kidney stone formation and prevention has never received sufficient attention. We therefore felt that a more systematic study of the action of hydrophilic colloids might offer valuable information on how the formation of urinary concretions could be completely avoided or at least stopped. Such knowledge might even lead to the development of a more efficient method by which some stones could be removed without surgery.

From a colloid-chemical point of view urine must be considered as a supersaturated solution of extremely complex composition. The electrolytes and nonelectrolytes in the urine of a healthy person remain in solution at a much higher concentration than their solubility in pure water would indicate; this is due to the presence of protective colloids. This fact was stated by Lichtwitz some time ago (*4, 5*). Ord (*6*) and Rainey (*7*) had already observed that crystals formed from solutions containing hydrophilic colloids differed pronouncedly in their morphology and properties from those derived from pure aqueous solutions. To Ebstein (*2*) must go the credit for having been the first to draw attention (in 1884) to the importance of colloids in kidney stone formation. Since then much research pertaining to the etiology of stones has referred to an "unbalance" of colloids and crystalloids, but there is a serious lack of infor-

mation concerning further work on this phenomenon.

It would seem that colloids are a very important, yet often neglected, factor which deserves far more attention than it has received so far in the study of stone formation and prevention. We therefore decided to study systematically the concentration of hydrophilic colloids in the urine of several hundred individuals, both males and females, of various races, living in different climates, and to correlate the results with the occurrence of kidney stones in these individuals. These tests were carried out on various islands in the Pacific Ocean and also in west Florida, which is recognized as an area of high stone incidence (*8–10*).

Systematic colloid-chemical studies, which included surface tension determination, ultramicroscopic investigation, determination of electric charges carried by the colloidal particles visible in the specimens, and chemical analysis of the urine samples offered definite proof that the urine of individuals giving evidence of stone formation was deficient in protective colloids, and that this was the main factor in stone formation. As a matter of fact, it could be ascertained that the incidence of stones in both sexes in different ethnic groups and nationalities is inversely proportional to the protective urinary colloids present. It was also found that the urine of females generally has a higher concentration of hydrophilic colloids than that of males. It was also demonstrated that the concentration of ultramicroscopically visible protective colloid particles in the urine of pregnant women is larger than that in the urine of nonpregnant women.

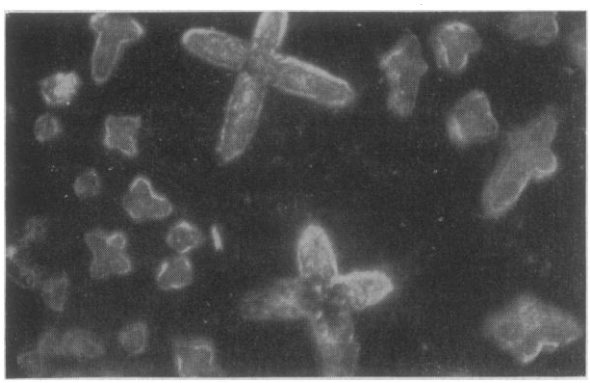

FIG. 1. Urine passed by patient prior to treatment. Photo-ultramicrograph of extremely alkaline urine of white male due to urea-splitting organisms. Multiple bilateral rapidly recurring stones. × 1200.

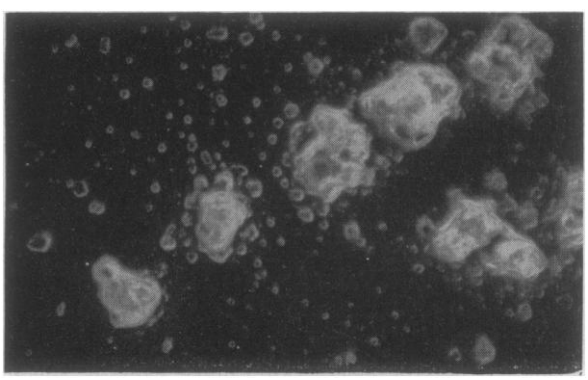

FIG. 2. Urine passed by same patient 1 hr after parenteral injection of 300 TRU of hyaluronidase mixed with physiological saline. × 1200.

All surface tension determinations were carried out by the pendent drop method (*11*), the only method that can offer reliable results when studying solutions as complex as urine. The results obtained with the urine of white females gave an average of 60 dynes/cm, whereas the average with specimens from Negro females amounted to only 52 dynes/cm. In contrast, the corresponding figures for males were 65 and 55 dynes/cm, respectively. The surface tension of specimens from a Negro female dropped from 52 to 42 dynes/cm from her second to her seventh month of pregnancy.

The correlation of these results with the tendency to stone formation would indicate that from a colloid-chemical point of view the presence of capillary-active agents might be the predominant factor because of their action as protective colloids and dispersing agents, forming a reversible gel in combination with the crystal micelles, instead of permitting the micelles to grow into solid crystals of inorganic matter.

It was therefore decided to ascertain whether the concentration of protective urinary colloids could be increased by parenteral injections of a potent protective colloid and dispersing agent. From a biochemical point of view the injection of Wydase® (150 TRU)[1] dissolved in 1 ml saline solution seemed to be of special interest because of its high capillary activity and pronounced protective action. This preparation is hyaluronidase, an enzyme with a fairly high molecular weight. Hyaluronic acid, one of the two substrates of hyaluronidase, is a complex mucopolysaccharide compound, of very high molecular weight, composed of alternating units of acetyl glucosamine and glucuronic acid. Extensive ultramicroscopic studies had already offered visual proof that it is the hyaluronic acid component which acts as a strong peptizing agent and protective colloid. Figs. 1 and 2 offer visual evidence both of the effectiveness of injecting the enzyme as produced by the Wyeth Institute of Applied Biochemistry and of its action.

We feel that this discovery of the action protective colloids exert in preventing the formation or development of stones might well open up a new and hitherto neglected field for medical science far beyond the treatment for kidney stones. This statement is based on recent observations which indicate that the formation of protective colloids in the human body virtually disappears during times of strong emotional stress.

Urine is a highly concentrated solution due to the presence of certain colloids. The protective action of urinary colloids is of major importance in preventing

[1] One TR (turbidity-reducing) unit of hyaluronidase is the amount which will, in 30 min, reduce the turbidity produced by 0.2 mg hyaluronic acid (mixed with acidified serum) to that produced by 0.1 mg, under standard conditions.

precipitation, agglomeration, and conglomeration of crystalloids from such a solution.

If the concentration of such protective colloids is insufficient, stone formation begins or is accelerated. In 680 subjects the incidence of stone was found to be almost inversely related to the degree of protective urinary colloids present.

Subcutaneous injection of hyaluronidase (mixed with saline) causes a pronounced increase in protective urinary colloids. The colloids may form a gel and thereby prevent crystallization of the electrolytes present. They act as excellent dispersing agents, preventing the formation of stones.

Hyaluronidase therapy has been effective in preventing the formation or recurrence of urinary calculi over a period of 11–15 months in 18 of 20 patients with a tendency to rapid stone formation.

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Multicurie Cobalt 60 Units for Radiation Therapy

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One of the fundamental objectives in the use of radiation in cancer therapy is to deliver a large dose of radiation to the tumor with a minimum dose to the healthy tissue. When the tumor is situated below the skin surface it is necessary to use radiation which gives a high-percentage depth dose. This percentage depth dose is defined as the ratio of the dose received at a depth below the surface to the maximum dose which occurs at or near the surface. Two important factors affect this percentage depth dose: (*a*) the distance from the source of radiation to the skin (SSD), and (*b*) the energy of the radiation. If the SSD is 10 cm, then the dose received at a point 10 cm below the surface of the skin cannot be greater than 25% in accordance with the inverse square law for a diverging beam. In practice, the percentage depth dose is even less than this, because of the absorption of the radiation. As the energy of the radiation is increased, the beam becomes more penetrating, and more radiation is delivered to the underlying layers of tissue.

When radiations of energy greater than 1 mev interact with matter, most of the electrons are projected in the forward direction, so that the energy absorbed in the superficial layers of the skin is less than that absorbed a few millimeters below the surface. For the radiation from Co^{60}, the maximum dose is delivered 5–6 mm below the surface. This is another advantage of high-energy radiation.

For many years large sources of radium (5–10 g) have found limited usefulness in the treatment of certain types of cancer. The dosage rate from these units is small, being about 10–12 r/min at a point 10 cm from the source. These units cannot be used at greater distances because of the low dosage rate, and so the percentage depth dose achieved is very small, being about equivalent to what can be obtained from a low-voltage x-ray machine. Nevertheless, because of the nature of the radiations, radium units have been used successfully in treating cancers where cartilage is involved. For these radiations, 1 g of fat will absorb about the same amount of energy as 1 g of bone, in contrast with the case of low-energy radiation where bone may absorb up to 10 times as much energy as the fat.

If a source of radium is made much larger than 10 g it becomes so thick that much of the radiation is lost by self-absorption within it. Aside from cost, this places an upper limit on the effective strength of such a unit. If the source is increased in area it is difficult to obtain a suitable beam of radiation.

In the Canadian reactor with its high neutron flux density, it is possible to produce sources of Co^{60} with specific activities of 20–60 curies/g in a reasonable length of time. One g of Co^{60} with an activity of 20 curies will give the same radiation output as 32 g of radium. It is therefore obvious that physically small sources with high activity are feasible using radioactive cobalt. With this higher activity, they may be used at larger SSD. The radiations from cobalt consist of 2 γ-ray lines of 1.17 mev and 1.33 mev, emitted in equal numbers as Co^{60} decays to Ni^{60}. The average energy of these is comparable to the energy of the radiations from radium sources.

In the summer of 1951 two sources of Co^{60}, each with an effective strength of 1000 curies, and having a specific activity of about 20 curies/g, were made available from the Canadian heavy water pile at Chalk River, for teletherapy units. One of these sources was installed in the University Hospital in Saskatoon, and the other in the Ontario Cancer

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