

dose of 120 mg in the third. In each instance there was an immediate increase in the excretion of pregnandiol. The effect of this hormone disappeared within 24 hours after the last injection (Fig. 6, 7, 8).

If the increase in pregnandiol above the average level excreted in each patient was calculated, about 30 to 35% of the progesterone injected could be accounted for as pregnandiol. Thus, in Fig. 6, 150 mg of progesterone was injected over a 3-day period and at least 50-60 mg of additional pregnandiol was recovered from the urine during this period. In the nonpregnant individual in the preovulatory phase it is possible to recover less than 10% of the progesterone as pregnandiol. The activity of the corpus luteum in the postovulatory phase and in the first trimester of pregnancy must exercise some effect on the metabolism of progesterone so that a much greater percentage can be accounted for as the inert metabolite, pregnandiol. It was also observed that a higher percentage of progesterone is recovered as pregnandiol when the steroid is administered in small divided doses than as a single large dose (Fig. 8).

Discussion. Diethylstilbestrol and testosterone propionate do not influence the excretion of pregnandiol during the first 16

weeks of normal gestation. On the contrary, progesterone is metabolized efficiently so that at least 30 or 35% is recovered as pregnandiol. If it is desirable to increase the amount of progestational hormone available during early pregnancy, it is more logical to administer progesterone in liberal amounts rather than diethylstilbestrol. However, it is the impression of the authors that there is no lack of progesterone in most of the pregnancies which threaten to end during the early months. Decreased pregnandiol levels may be an index of poor placental function.

Summary. Diethylstilbestrol, testosterone propionate and progesterone were administered to 15 patients with normal pregnancies in order to study the effect of these substances on progesterone metabolism and pregnandiol excretion. Diethylstilbestrol and testosterone when administered daily in large amounts over long periods during the first 16 weeks of gestation did not alter the normal excretion of pregnandiol. The administration of progesterone was followed by the prompt recovery of a considerable portion of the injected steroid as pregnandiol.

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A Gelatinous Variant of *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa is generally recognized as highly variable. Among the variations exhibited are those of colony form. During the course of cultural examinations made on a case of third-degree burn in 1943, a variant was isolated which seems not to have been described before. It appeared in small numbers as an unusual colonial form when the organism was streaked on a modified Sabouraud's medium. The modification

of this medium consisted of the addition of a 0.5% glycerol to the usual Sabouraud's maltose-peptone agar. Subsequent observations showed that the appearance of this variant depended on the presence of the glycerol in the medium.

The initial observations were as follows: among the usual colonial forms a small number of exceptional colonies were recognized on a few of the plates. These were few

enough to be sparsely scattered even in the more heavily seeded portions of the plate, but occurred here and there as well-separated colonies. They differed strikingly from the ordinary colonies and in young cultures resembled small pearls so closely that we have since referred to the variant as the "pearl form" of *Ps. aeruginosa*. Variations have been observed, however, within the form itself which make the term "pearl form" less appropriate for it than that of "gelatinous variant."

A remarkable feature of this variant, when grown on a solid medium containing glycerol, is its tendency to increase in size long after ordinary colonies have ceased to grow. If recently seeded plates are inverted and placed under a bell-jar at room temperature, growth of the individual colonies will usually continue until they have reached a diameter of a centimeter or more and a depth of several millimeters. Some eventually touch the cover of the inverted Petri dish and the more fluid of these variants often drip material onto the cover of the inverted dish. In general, however, they have a stiff jelly-like consistency and tend to preserve their form for many weeks. They become markedly elevated even when grown on upright plates. If the variant is seeded to a solid glycerinated medium by merely touching the surface of the medium at given points with an inoculating needle ("point inoculation"), exceedingly large mounds of growth are often produced. The colonies are translucent and glistening by reflected light and opaque by transmitted light. They differ in color. Smears show that virtually all of the colony consists of this gelatinous material, with relatively few organisms scattered through it. Since the organisms are much more numerous at the base of the colony, it would appear that the material is built up chiefly by the metabolic activity of organisms next to the surface of the medium. Stained preparations show that the material is acidophilic and drawn out in more or less heavy strands, many of which are stretched around perfectly clear areas, giving the mass an alveolar structure. Many of the broken strands are distinctly wavy and resemble elastic fibers

in animal tissue. Although much of it occurs in strands, the general physical appearance of the material is not that of a mucoid substance.

Growth on liquid media containing glycerol is also abundant and definitely gelatinous. The jelly-like layer formed at the surface of the medium, where *Pseudomonas* grows best, may become more than a centimeter in thickness. The floating mass hangs together well and exhibits no stringy or mucoid properties.

We have isolated a number of variants within this gelatinous type since we first isolated the "pearl form." It is well known that ordinary strains of *Pseudomonas* may show many variations in colony form; also variations in pigment production. Many of the variations seen in ordinary nongelatinous colonies have counterparts in the gelatinous variant. Those first isolated were chiefly of a milky or pearly-white color; those isolated later, either as new gelatinous forms or as variants of the original pearl forms, range from amber through red to green. They differ also in form. The "pearl forms" initially isolated had evenly rounded surfaces; however, many of those isolated since from the same strain of *Pseudomonas* show gently sloped irregularities in their surface contours much as though the colony consisted of intertwined ropes of gelatinous growth. Some develop a long gelatinous nipple or tongue-like upgrowth from the center of the colony. There are other departures from an evenly rounded surface. These differences have been associated with basic differences in colony form observed among ordinary nongelatinous colonies of *Pseudomonas*. A common nongelatinous type is a low convex or pulvinate colony with a rounded surface studded or not with small papillae and with or without a thin spreading edge. Another type is relatively flat and more or less villous by virtue of interlacing strands or cords of more elevated growth drawn through the colony. This colonial form has been described in the literature as a "rugose" form.¹ Ap-

¹ Fiala, S., *Centralbl. f. Bakt. u. z. w., Abt. I*, Orig., 1941, **148**, 58.

parently the more evenly rounded "pearl form" is a gelatinous variant of the first-mentioned type of nongelatinous colony; the second a gelatinous variant of the ordinary rugose form. One may also observe among ordinary colonial forms, whether rugose or not, those which have definitely raised centers ("umbonate" colonies). The latter seem to give rise to gelatinous forms with the long nipples or tongue-like processes referred to earlier. These conclusions are based on observations which have been made on gelatinous variants seeded to glycerol-free media. When this is done the gelatinous variant reverts to the nongelatinous type. Although quite stable on glycerinated media, there is nevertheless some tendency for nongelatinous variants to arise from the gelatinous ones. This tendency becomes more evident after the variant has been grown for a time in ordinary broth and is then transferred back to a solid medium containing glycerol. Under these conditions there are usually a few colonies which are not of the gelatinous type.

From the observations related above it appears that what we have here is a purely physiological variation, one involving ability to utilize glycerol for the formation of the particular gelatinous material, this functional variation not being associated with any one basic colony type. It may, in other words, be superimposed upon any one of the various colony forms known to be produced by this organism. The fact that very few colonies among the many which were originally iso-

lated showed this peculiar physiological ability speaks for its rarity. It also seems infrequent among other strains of *Ps. aeruginosa*. So far we have been able to relate the property only to the utilization of glycerol, although the possible effects of other polyhydric alcohols are still to be investigated, likewise the nature of the gelatinous material produced.

Since a strong pyocyanus phage was isolated from mixed broth cultures of the exudate from the lesions, it was thought possible that the variation in question might have arisen in some way from exposure to phage. The gelatinous variants, however, proved quite as susceptible as the nongelatinous forms to the phage we isolated. Moreover, filtrates of a number of broth cultures of these gelatinous variants failed to show that they themselves carry phage.

Summary. What appears to be a new variant of *Pseudomonas aeruginosa* is described. It is characterized by the production of large amounts of gelatinous material when grown on media containing glycerol, and on solid media gives rise to markedly raised colonies of a stiff jelly-like consistency and to a thick gelatinous layer near the surface of liquid media. The variation seems to be entirely one of ability to utilize glycerol for the production of the particularly gelatinous material, and to be unrelated to any one of the several basic colony forms known to occur in this species.