Summary. 1. The serum protein bound radioactive iodine (I^{131}) level was determined 24 hours after the oral administration of 150 microcuries I^{131}. Twenty subjects were studied; 10 were thyrotoxic and 10 euthyroid. The protein bound I^{131} was determined by a modification of the method described by Chaikoff *et al.*[8]

2. The serum protein bound I^{131} in thyrotoxic patients was 38 to 146 $\times$ 10^{-5} microcuries/cc averaging 68. In euthyroid subjects, the serum level ranged from 3 to 28 $\times$ 10^{-5} microcuries/cc, averaging 13.

3. It would appear that this test may be of diagnostic value as a measure of thyroid function.

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Inactivity of Bound Plasma Progesterone.*

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Observations have been reported indicating that progesterone in blood is in the plasma and not in the cells and that most of the total progesterone is free while rarely more than 10% is bound to protein or some other substance or substances that render this portion of the progesterone insoluble in acetone and ether.[1] The latter circumstance contrasts with the finding that approximately two-thirds of the estrogen in blood is bound.[2-4] It has been suggested that bound estrogen serves in effect as a reservoir, that the estrogen readily dissociates at the cell membrane, and that the bound fraction is potentially active estrogen.[3] Several observations indicate that a comparable situation does not obtain with respect to bound progesterone. The relative amount of the bound fraction seems too small to serve as a significant source of free progesterone; when introduced directly into the uterus of the mouse the bound fraction had no effect in a test period of 48 hours, presumably sufficient time to permit dissociation if it is to occur; the activity of raw plasma is identical with that of the free progesterone it contains. In short, bound progesterone appeared to be biologically inert.

When progesterone is introduced into the spleen or portal vein and must pass through the liver before reaching the systemic circulation the absorbed progesterone is inactivated in the sense that no effects of the substance are observed in rats,[5] rabbits,[6,7] and mice.[8] If the blood of such animals should contain levels of bound progesterone that would be effective if free, it would constitute further and probably strong evidence for the biological inactivity of the bound fraction. Such an experiment is described here.

Experimental. Young adult mice were used as test animals; the inbred A strain of Strong[9] was chosen to minimize variability. Of a group of 22 animals ovariectomized 13 days earlier, seven were given progesterone pellets subcutaneously, eight were given progesterone

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pellets intrasplenically, and 7 were reserved as untreated controls. Of a group of 20 mice ovariectomized 47 to 49 days earlier, ten were given progesterone pellets subcutaneously, and 10 were given progesterone pellets intrasplenically.

The pellets were made of crystalline progesterone‡ and weighed 1 to 3 mg. Each animal received a single pellet. The subcutaneous pellets were implanted on the flank by means of a trocar and without anesthesia. The intrasplenic pellets were implanted under sodium amytal-ether anesthesia; splenic hemorrhage was controlled by packing wedges of muscle in the wound.

Four days after implantation of the pellets the mice ovariectomized at the beginning of the shorter period were bled by cardiac puncture. The blood from the members of each of the three subgroups (untreated, with subcutaneous pellets, with intrasplenic pellets) was pooled. Six, 8, 12, 16 and 22 days after implantation of the pellets two mice ovariectomized at the beginning of the longer period and carrying intrasplenic pellets, and 2 carrying subcutaneous pellets, were bled by cardiac puncture. The bloods from mice with the same histories were pooled. Sodium citrate was employed as the anticoagulant. Each pooled sample was centrifuged, and the free and bound fractions of progesterone in the plasma were separated by modification[1] of the method of Szego and Roberts[10] for separation of free and protein-bound estrogen. Briefly, the method involved precipitation of the plasma proteins with 10 volumes of acetone in the cold. The acetone solution contained the free progesterone, and the bound progesterone was freed by partial acid hydrolysis of the proteins. The two fractions were dissolved in sesame oil, and the progesterone content of each was assayed by intra-uterine injection in mice, a method that detects 0.0002 μg.[11]

After bleeding each mouse was autopsied, and the uterus was fixed in Lavdowsky's fluid[12] and prepared for microscopic study.

Findings. The pellets were intact and correctly located. No splenic adhesions were encountered in the animals carrying intrasplenic pellets. The uteri of most of the mice with subcutaneous pellets were of varying shades of yellow and slightly but distinctly enlarged; the uteri of the mice that had carried intrasplenic pellets for 22 days also had this appearance. The uteri of the other treated mice were like those of the untreated animals.

Microscopically, the endometria of the untreated mice showed no evidence of the action of progesterone; the stromal nuclei were shrunken, fusiform, and dense. Similarly, the mice carrying intrasplenic pellets showed no effects of progesterone upon the endometrium. The endometria of all of the mice carrying subcutaneous pellets for eight days or longer showed characteristic progestational changes in the stromal nuclei; they were enlarged, spherical, vesicular, and had distinct nucleoli.[13] When the subcutaneous pellets had been in place only four or six days no change in the stromal nuclei from the castrate condition was evident.

Assays of plasma from the untreated castrates revealed no free progesterone and at most a trace of bound progesterone.

The values for plasma progesterone in the mice carrying pellets are shown in Fig. 1. The animals carrying subcutaneous pellets had plasma levels that increased with the length of time the pellet was in place; indeed, the total progesterone levels (the sum of the free and bound fractions) fluctuated about a straight line when plotted against time. The level of free progesterone attained before an endometrial response was given was roughly 1.0 μg per ml.

The animals with intrasplenic pellets, on the other hand, had consistently low levels of free progesterone, the highest being 0.7 μg per ml. The bound progesterone, however, soon reached a high level in these animals, 2.0 μg per ml by the eighth day after implantation of

‡ The progesterone was generously supplied by Dr. Erwin Schwenk of the Schering Corporation.

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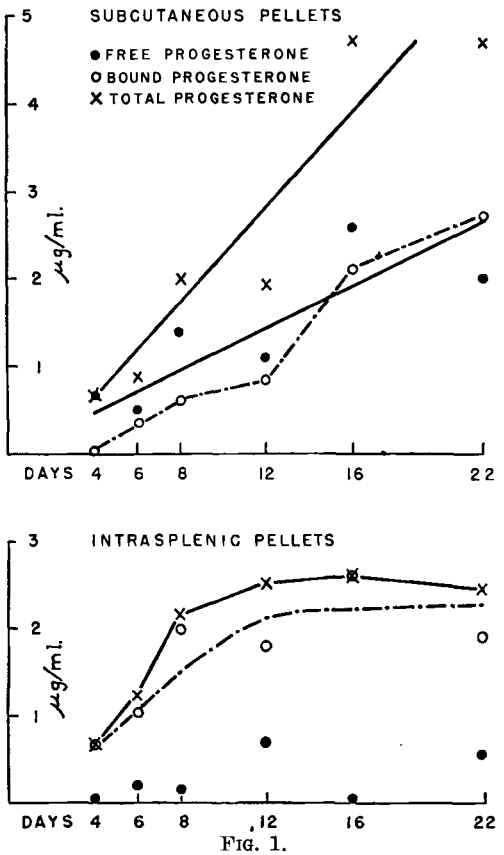

FIG. 1.

Plasma levels of free, bound, and total progesterone in ovariectomized mice with subcutaneous pellets and intrasplenic pellets of progesterone.

the pellets. Here the total plasma progesterone, in contrast to that of the animals with subcutaneous pellets, reached a plateau on the eighth day.

Discussion. All of the observations to date are in agreement in indicating biological inactivity of bound progesterone. The level of this fraction has been consistently low in various ovarian states;[1,14] amounts that would be effective if free have had no effect upon the endometrium when injected directly into the uterus of the mouse;[1] the effectiveness of raw plasma has been the same as that of the free progesterone it contains;[1] in the present experiment circulating levels of bound progesterone that regularly elicited endometrial responses when free were without effect even when present for presumably as long as 16 days.

The identity of the substance to which bound progesterone in the plasma is attached is unknown. It may be protein, as believed for estrogen.[2,3] On the other hand, the method employed for fractionation would probably not distinguish protein-bound material from conjugated material, such as a sulphate or a glucuronide, inasmuch as such conjugants would presumably be precipitated along with the plasma proteins by excess acetone. Whatever its nature, the binding appears to be firm and to dissociate slowly. This is suggested by the lack of response when bound progesterone is injected directly into the uterus and the endometrium is examined 48 hours later, presumably an adequate time for dissociation if it is to occur.[1] No or slow dissociation is further indicated by the present observation that levels of bound progesterone as high as 2.7 μg per ml may be present in the plasma for presumably as long as 16 days without dissociation of enough free progesterone to evoke a recognizable response in the endometrium. Accordingly, binding seems to serve no useful function in the circulation or availability of progesterone.

On the other hand, binding of progesterone is at least one of the methods of inactivating this hormone when it is absorbed in the spleen of the mouse. These observations do not show whether the binding and inactivation occurred in the liver or in the spleen, or indeed in the portal vein. Other studies,[5-7] however, have shown that the liver can inactivate this steroid, and have not revealed appreciable inactivation by the spleen. So far as we are aware, hepatic inactivation of steroids has been found in the past to involve alteration of the steroid compound.[15-20] Whether bind-

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ing of an apparently otherwise unaltered steroid compound is a method of inactivation peculiar to progesterone has not been determined.

No explanation can be offered at present for the apparent plateau in bound and total progesterone beginning eight days after implantation of the intrasplenic pellets. Obviously, the rate of absorption may have stabilized or declined, or the rate of removal of bound progesterone from the circulation by excretion or chemical alteration may have increased. Another possibility is that of increasing hepatic inactivation of the progesterone as absorbed by means other than binding.

The apparently linear relation of free and total plasma progesterone to time after the implantation of subcutaneous pellets also raises problems. One of the more interesting of these is the increase in the level of bound progesterone in these animals, possibly suggesting eventual hepatic inactivation of part of the absorbed material by binding. Although it may prove to be of no significance, it is interesting that the maximal level of bound progesterone was almost identical in the two groups of animals.

An interesting fact that emerges is that the minimal physiologically effective plasma level of progesterone with respect to the endometrium appears to be of the order of 1.0 μg per ml of the free fraction. Every animal with this or a higher level exhibited progestational changes in the stromal nuclei. All animals with lower plasma levels showed no endometrial response, irrespective of the level of bound progesterone.

Summary. Pellets of crystalline progesterone implanted into the spleens of ovariectomized mice had no effect upon the endometrium despite the presence of relatively high levels of bound progesterone in the plasma; the highest level of free progesterone in these animals was 0.7 μg per ml. Subcutaneous pellets of progesterone produced characteristic progestational changes in the endometrium when the level of free plasma progesterone exceeded 1.0 μg per ml. Binding appears to be a mechanism of hepatic inactivation of progesterone in the mouse.

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Effect of Dialyzed Enterogastrone Upon Twelve-Hour Nocturnal Gastric Secretion in Man.*

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Previous studies[1] have demonstrated occasional decreases in the 12-hour (nocturnal) and 24-hour gastric secretion in man following the intramuscular injection of 1000 to 3000 mg of an enterogastrone concentrate. This effect was variable in degree and temporary in duration. The administration of 400 to 2000 mg did not alter significantly the secretory response of the human stomach to the single standard dose of histamine or to the repeated injection of small amounts of histamine.[2] Insulin-stimulated secretion likewise was not affected, although the response possibly was modified by doses of 2000 or 3000 mg. Ferayorni, Code, and Morlock[3] similarly noted no change in gastric secretion during a double histamine test in 10 human subjects given 200 mg of enterogastrone intramuscularly. In 14 volunteers, quantities up to 400 mg intramuscularly and 18 g orally

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