TABLE III. Effect of Injections of Boiled Anemic Rabbit Plasma Filtrate or Saline on Blood Values in Rats Fed Cobalt.

Treatment	Initial	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk
	Hct %							
Cobalt and saline	44.4	44.8	45.7	50.8*	53.6*	53.5*	55.3*	56.0*
Cobalt and boiled filtrate	44.8	48.0*‡	50.5*‡	53.5*	52.3*	59.4*	57.0*	62.2*‡
Saline	44.0	44.0	44.0	41.0	44.0	43.9	45.2	44.1
Boiled filtrate	43.6	47.6*	47.3*	48.3*	50.0*	49.4*	47.2†	50.3†

Each figure represents avg of 5-7 rats.
* Significant difference from saline control at 1% level.
† *Idem* 5% " .
‡ " cobalt and saline group at 1% level.

lus was removed, hematocrit values of the saline injected group returned to normal. On the other hand, after return to sea level pressure the hematocrit percentages of rats which received boiled anemic plasma remained above those of the saline injected controls.

Cobalt feeding. Feeding 0.01% cobalt produced a polycythemic response in normal rats as indicated by an increase in hematocrit value (Table III). When boiled anemic rabbit plasma was administered in conjunction with the cobalt feeding an additive effect was observed.

Discussion. Boiled anemic rabbit plasma filtrate containing erythropoietin as well as anemia, hypoxia and cobalt feeding stimulate erythropoiesis. However, when prolonged erythropoietin administration is combined with either anemia or hypoxia, no additional effect is noted. Possibly the endogenous erythropoietin produced by hemorrhage or hypoxia exceeds and masks the effect of the relatively small quantity of exogenous and possibly partially denatured material injected.

The mode of action of cobalt in producing polycythemia is unknown. Unlike its effect in anemia or hypoxia, erythropoietin in boiled filtrate has an additive action in cobalt polycythemia which is evident in the early stages of combined stimulation. This result may be explained by assuming that the cobalt stimulus is suboptimal or that a different mechanism is activated by cobalt.

Summary. 1. Repeated administration of erythropoietin fails to increase the erythropoietic response to hemorrhage or hypoxia, measured by hematocrit or reticulocyte values. 2. Polycythemia is greater after simultaneous administration of cobalt and erythropoietin than after cobalt feeding alone.

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Localization of Fluorescent Antibodies to Human Growth Hormone in Human Anterior Pituitary Glands.* (25790)

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The antigenicity of human growth hormone (H.G.H.) has now been clearly established (1,2,3,4). With the advent of more highly purified preparations of H.G.H. the specificity of the corresponding antisera has been supported by studies using hemagglutination-inhibition(1,2,4) and agar gel-diffusion(3) and biological inhibition technics(3). Using

the fluorescent antibody technic, Marshall (5) showed that antisera to hog ACTH localized in basophil cells of hog anterior pituitary glands. In their more highly controlled study, Cruickshank and Currie(6) were able to localize fluorescent antisera to human ACTH, TSH and gonadotropin in basophil cells of human pituitary glands. However this localization could not be inhibited with unlabelled antiserum or absorbed out by prior incubation with the specific antigen, and was therefore considered non-specific. Fluorescent antisera to human growth hormone localized in other anterior pituitary cells which were not identified. This fluorescent staining could be absorbed out by prior incubation of the antisera with H.G.H., and with ACTH and TSH as well, but could not be inhibited with unlabelled anti-H.G.H. They attributed these results to lack of purity of their hormone preparations, resulting in a lack of specificity of the corresponding antisera as demonstrated by agar gel-diffusion and precipitation-absorption technics. In this study we have attempted to examine further the specificity of our antisera to human growth hormone by investigating their localization as fluorescent conjugates in human anterior pituitaries. We hoped furthermore to demonstrate thereby the cellular site of production or storage of human growth hormone in the human pituitary gland.

Methods. Antisera to H.G.H. were prepared by immunizing rabbits with Raben H.G.H.(7). These antisera were tested for antibody titer and specificity(1). Rabbit anti-human gamma globulin (Cohn fraction II) and goat anti-rabbit gamma globulin were similarly prepared. Fluorescin isothiocyanate was prepared according to Riggs(8) and coupled to immune gamma globulin according to Marshall(9). The conjugates were absorbed with mouse liver powder prior to use (10). Pituitaries used for specificity studies were obtained at autopsy less than 2 hours post mortem. They were frozen in liquid nitrogen and stored at minus 20°C. Sections were prepared according to the method of Vasquez (11). They were not washed prior to fixing as this washes the parenchymal cells out of the gland. Pituitaries and pituitary adenomata used to demonstrate specific cellular localization were formalin-fixed paraffin-embedded blocks. Four micron sections were cut and deparaffinated in the routine manner except that they were not exposed to formaldehyde vapor. Staining of sections with the fluorescent conjugates and inhibition control studies were performed as described by Vasquez(11). All slides were mounted in buffered glycerine and studied with a Leitz fluorescence microscope unit. The formalin-fixed paraffin-embedded blocks were cut and mounted in pairs of adjacent sections. The first section of the pair was flipped over prior to being placed on the slide; the second section was mounted on the slide right side up so that the uppermost aspects of both sections were adjacent sides of the same interface and mirror images of each other. In this way we could be reasonably certain that we were looking at the same cell in both slides. The first slide was stained with the fluorescent conjugate and the second with either H and E

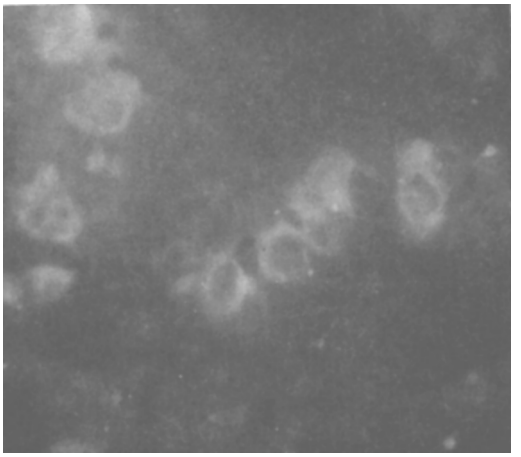

FIG. 1. Frozen section of human anterior pituitary stained with fluorescent anti-H.G.H. Note bright cytoplasm with dark nuclei. Scattered small bright spots are artifacts.

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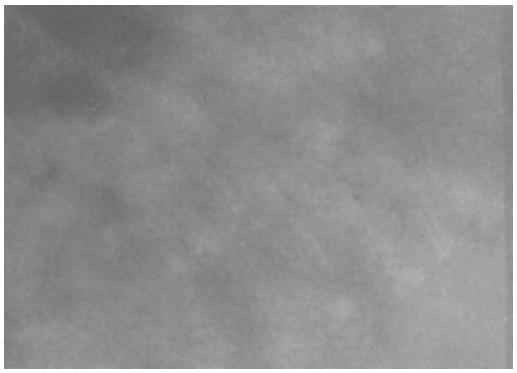

FIG. 2. Frozen section—human anterior pituitary (same as Fig. 1) stained with fluorescent anti-H.G.H. that has been absorbed with H.G.H. Note absence of specific staining.

or Aldehyde Fuchsin(12). Photomicrographs were made of identical fields. Those of the fluorescent sections only were printed using the reverse side of the transparency, so that the resultant prints would be superimposable.

Results. Frozen and formalin-fixed sections of normal human anterior pituitaries stained with fluorescent anti H.G.H. showed bright apple-green fluorescence in groups of cells and single cells (Fig. 1, 4). Unstained sections showed no fluorescence. There was no specific staining in the posterior pituitary, brain, parathyroid gland, thyroid gland and nasal polyps. Background fluorescence was higher in all of the formalin-fixed sections.

The fluorescent staining was markedly reduced in the frozen sections and completely absent in the formalin-fixed sections when stained with fluorescent anti-H.G.H. that had been previously absorbed with H.G.H. in the test tube (Fig. 2).

In the paired inhibitions studies, localization of fluorescence in anterior pituitary cells was not inhibited by prior incubation of the slide with non-fluorescent anti-human or anti-rabbit gamma globulin, but was much reduced in the corresponding slide which had been incubated with non-fluorescent anti-H.G.H. The frozen sections of human pituitaries did not stain with goat anti-rabbit gamma globulin. When stained with fluorescent anti-human gamma globulin they showed bright fluorescence in the walls of blood vessels and supporting framework but not in the parenchymal cells (Fig. 3).

Two eosinophilic adenomata, from patients with acromegaly showed specific staining in all the adenomata cells (Fig. 6). The staining was absent when stained with fluorescent anti-human growth hormone previously absorbed with human growth hormone. Chromophobe adenomata from patients with no history of acromegaly showed no fluorescence in any of the cells when stained with fluorescent anti-H.G.H. An unclassified pituitary adenoma from a patient with gross Cushings' Disease showed no specific staining of adenomata cells.

When mirror images of adjacent sides of the same interface (one stained with anti-human growth hormone, the other with the Gomori aldehyde fuchsin method) were studied, it was seen that the fluorescence was localized in most but not all eosinophil cells. It was not possible in these slides to detect differences between those eosinophils which showed fluorescence and those which did not. With very few exceptions (less than one cell per hundred cells studied) there was no fluorescence in cells other than eosinophils (Fig. 4, 5).

Discussion. Previous studies attempting to localize specific anterior pituitary hormones in pituitary glands by the fluorescent antibody technic(5,6) have been hampered by lack of purity of the hormone preparations used as antigens. With the use of a more highly purified H.G.H. preparation now available a specific antiserum can be obtained.

Our studies further indicate that these an-

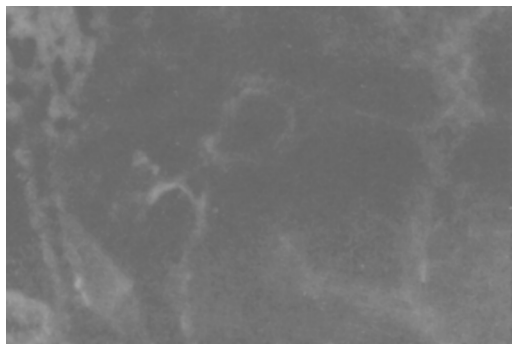

FIG. 3. Frozen section—human anterior pituitary stained with fluorescent anti-human gamma globulin. Note staining of fibrous framework and absence of staining in parenchymal cells.

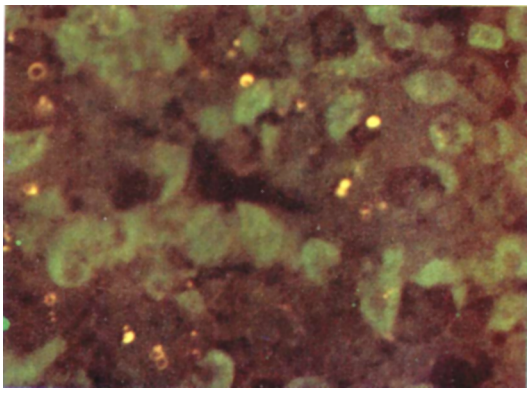

FIG. 4. Formalin-fixed section—normal human anterior pituitary stained with fluorescent anti H.G.H. Note bright green fluorescence in some cells. On comparison with Fig. 5 these cells can be seen to be acidophils.

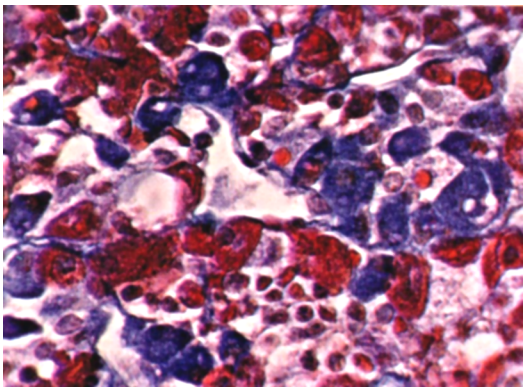

FIG. 5. Adjacent section to Fig. 4—stained with the Aldehyde-fuchsin method. Basophil and chromophilic cells correspond to non-fluorescent areas of Fig. 4.

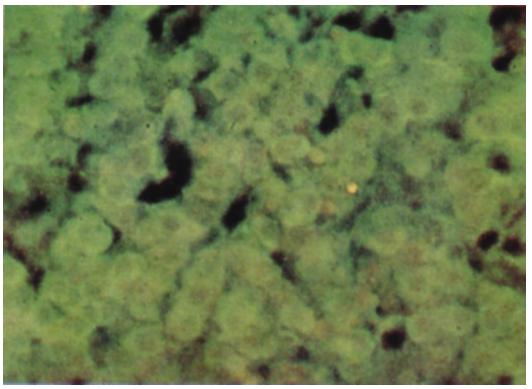

FIG. 6. Eosinophilic adenoma from a patient with acromegaly stained with fluorescent anti H.G.H. All adenoma cells are brightly stained.

tibodies react not only to human growth hormone preparations which had been used as antigen, but also with an antigen located in eosinophil cells of human anterior pituitaries and eosinophilic adenomata from patients with acromegaly. The specificity of the reaction is demonstrated by the staining of anterior pituitary cells exclusively with fluorescent anti-H.G.H. and by the following controls. This staining can be inhibited with unlabelled human growth hormone; it can be absorbed out with human growth hormone; it does not localize in tissues other than human anterior pituitary glands and eosinophilic adenomata, and fluorescent antisera of heterologous nature do not localize in the parenchymal cells of the human pituitary gland.

These experiments strongly suggest that human growth hormone is either produced or stored in most, but not all, the eosinophil cells of the human anterior pituitary. The very few exceptions to the exclusive localization of the fluorescent anti-human growth hormone in the eosinophil cells in the adjacent section studied is probably within the experimental error, but another explanation may be required. It is unlikely that the localization of fluorescence in the few basophil cells is due to a contaminating antibody or we would have expected more basophil cells to be stained.

Conclusions. Using the fluorescent antibody technic it has been shown that labelled rabbit antibodies to Raben human growth hormone localized specifically in cells of the human anterior pituitary gland. This antibody probably localizes exclusively in eosinophil cells of normal human pituitaries and in eosinophilic adenomata from patients with acromegaly.

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Distribution and Excretion of Radiofluoride in the Human.* (25791)

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During recent years there has been increased interest in fluoride metabolism particularly in regard to adoption of water fluoridation for partial prevention of dental caries(1). Because the fluoride ion is a trace element and occurs biologically in very small quantities‡ use of a radiotracer for human metabolism studies has many advantages. The only useful

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‡ i.e., human plasma fluoride values are of the order of 0.1-0.3 ppm(2).