altered significantly. Second, it is probable that the central core of DNA remains intact. This core, lying within a coat of denatured protein, is relatively stable to storage and to moderate heating, and it is unaffected by exposure to DNase. It is, however, readily destroyed by visible light in the presence of toluidine blue and by ultraviolet irradiation.

TAM is seemingly inert when studied alone *in vivo* and *in vitro*. Inoculated rabbits not only fail to develop any signs of infection, but also fail to develop immunity to a subsequent challenge with live myxoma virus. A question of interest is what happens to TAM in the presence of live fibroma virus. A first thought was that the live virus might function in promoting entry of TAM into tissue culture cells. Once inside, TAM particles would lose their outer coats and the central DNA would replicate itself. The action of fibroma virus, however, is probably more specific. Several experiments indicated that TAM particles can attach to and possibly enter cells alone and even a related virus, vaccinia, will not lead to transformation when given simultaneously. Fibroma virus sets up special conditions after infecting a susceptible cell. Febvre *et al.*(9) have shown that all recognizable fibroma virus disappears shortly after entry and an area of viroplasm, the inclusion body, appears within the next 5 hours. Virus particles begin to emerge from this area about 8 hours after infection. One wonders whether TAM particles coming to this matrix area might not lose their outer coats and then replicate themselves from the central cores of DNA. Investigations are being continued to better characterize the transformation process and the interactions involved.

Summary. The transforming agent prepared from myxoma virus (TAM) is resistant to heat, to ether extraction, and to mild enzymatic digestion but is inactivated by the photodynamic action of toluidine blue. TAM particles can be sedimented by ultracentrifugation (Spinco). TAM becomes attached to rabbit kidney cells in 15 minutes, when inoculated by itself, is not destroyed by incubation in tissue cultures for 48 hours at 36°C, and can be added before or after live fibroma virus in transformation experiments. The transforming agent is not immunogenic against myxoma virus in rabbits.

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On the Nature of Oxytocin and Vasopressin from Human Pituitary.* (24154)

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Comparison of pressor-antidiuretic hormones isolated in this laboratory from posterior pituitary glands of beef and hog has shown them to be cyclic octapeptide amides differing in one amino acid residue, the beef vasopressin containing arginine(1) and vasopressin from hog glands, lysine(2). The structure of arginine vasopressin, the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-arginylglycinamide, may be repre-

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sented as follows: CyS-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-CyS-Pro-Arg-Gly(NH_2). In lysine vasopressin, the lysine residue occurs in the place occupied by arginine in arginine vasopressin. On the other hand, oxytocins from these 2 sources appear to be identical and differ from vasopressins in having an isoleucine residue in place of phenylalanine and a leucine residue in place of arginine or lysine(3). Thus, interest was aroused in establishing the nature of the posterior pituitary hormones, particularly the vasopressins, from glands of other species. Van Dyke, Engel and Adamsons(4) recently noted a difference between pharmacological effects of lysine and arginine vasopressin, namely that the ratio of pressor potency to antidiuretic potency was 1:1 for arginine vasopressin, and 6:1 for lysine vasopressin when the antidiuretic potency was determined by intravenous injection in the hydrated, unanesthetized dog. They also tested for these relative potencies in extracts of pituitaries of man, monkey, dog, rat, sheep, and camel, and found a 1:1 ratio to exist between pressor and antidiuretic potencies. These results suggested that arginine vasopressin was the pressor-antidiuretic hormone in these species, although, as van Dyke and his collaborators pointed out, their conclusions were tentative and must be confirmed by isolation of the hormone from each species and determination of the amino acid composition of each vasopressin. Recently we reported a procedure for isolation of oxytocin and vasopressin from posterior pituitary glands of beef and hog which was applicable to small scale work(5). In this method the hormones are separated as a complex attached to a protein fraction and subsequently separated from proteins and then from one another in high yield. The method has now been adapted to the study of oxytocin and vasopressin from the human pituitary.

Methods. Human pituitary lobes were placed in acetone at the time of post-mortem examination, and later the posterior lobes were separated and pulverized to a powder.† Preparations of the powder varied in oxytocic and pressor activity from 0.2 to 0.35 unit/mg. Oxytocic activity was measured by the avian depressor method of Coon(6), and the rat pressor assay was used to determine vasopressor activity(7). A preparation starting with 3.3 g of human posterior pituitary powder provided a sufficient quantity of active components for their subsequent characterization. This amount of acetone-desiccated powder was extracted twice with 100 ml of 0.25% acetic acid, then with 50 ml of acid (at –5°C with stirring). The extraction times were 17, 8, and 18 hours, respectively. The combined extracts were lyophilized, then dissolved in 0.25% acetic acid to give a concentration of about 30 units/ml. Sodium chloride was added to final concentration of 10% and the protein precipitate was removed by centrifugation. The protein precipitate resulting from addition of sodium chloride was suspended in a minimum volume of water and dialyzed against water. The contents of the sack were acidified and centrifuged to remove a small amount of sediment and the solution was lyophilized. The residue was dissolved in 0.25% acetic acid and the proteins precipitated by addition of TCA to a final concentration of 10%. The precipitate was dissolved in 0.25% acetic acid and again precipitated with TCA. The last step was repeated and the combined supernatants were added directly to a column (0.9 x 14.5 cm) of IRC-50 (H^+), which was washed with 0.25% acetic acid until the pH of the effluent was the same as that of the acid. It was then washed with water. Trichloracetic acid was removed by the washes leaving peptides bound to the resin. The oxytocic and vasopressor activities were separated by gradient elution chromatography(8) with ammonium acetate buffers, 0.1 M, pH 5.0 → 0.5 M, pH 7.7 → 0.75 M, pH 7.7 (Fig. 1). The fractions were desalted on IRC-50 (H^+) columns and the eluted components were lyophilized. Yields and degree of purification obtained at different stages are presented in Table I. It will be noted that the hormones of the human pituitary could be purified in a manner similar to that described

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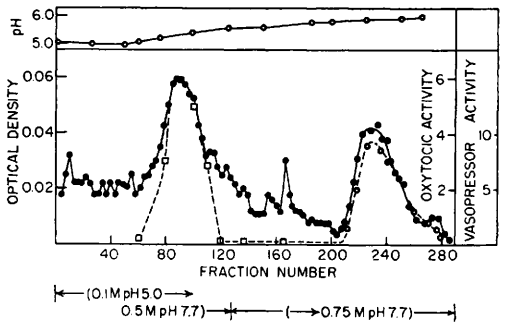

FIG. 1. Column separation of oxytocic and pressor principles. Resin, IRC-50; column, 0.9 × 14.5 cm; buffer, ammonium acetate; vol/tube, 1 ml; flow rate, about 2 ml/hr; optical density is of the Folin color on contents of every third tube. □, oxytocic activity; ○, pressor activity.

for beef and hog glands(5). Addition of 10% sodium chloride to the pituitary extract resulted in precipitation of a protein fraction containing the major part of the activity, although a greater part of the activity was soluble in this case than was true in the earlier work. However, the yield of the protein fraction was sufficient to permit further studies.

Results. Chromatographic behavior of the biologically active components of human posterior pituitary gland can be compared to previous results obtained for oxytocin and vasopressin derived from beef glands. In this system oxytocin is eluted at a pH of 5.3, and arginine vasopressin emerges at pH 6.0(5). The properties of human hormones on the column are those of oxytocin and arginine vasopressin. The agreement of the curve for the Folin color with that for biological activities was very good, indicating that the components were of high purity, particularly the vasopressin. No peptide material was still adsorbed on the column since Folin-positive material was not eluted after the column was washed with 4% ammonium hydroxide.

It should be pointed out that the yield of chromatographically separated components obtained from 3.3 g of acetone-dried pituitary glands amounted to 250 to 300 units of each activity. Since the effluent fractions from the chromatographic separation were analyzed by the Folin reagent, one-third of the activity was consumed in this process. The weight of material isolated after the column procedure represented a fraction of a milligram. Consequently, further work could only be of a qualitative nature for identification of the active components.

TABLE I. Purification of Oxytocin and Vasopressin from Human Posterior Pituitary Glands.

Step	Total activity (units) V*	O*	Folin color, %
Extraction of pituitary powder with acetic acid followed by lyophilization	974	683	100
Precipitation of protein complex followed by dialysis	414	369	31
Dissociation of complex and gradient elution chromatography	330	185	†
Desalting and lyophilization	174	68	†

* V = pressor activity; O = avian depressor activity.
† Not determined.

TABLE II. Comparison of Human Oxytocic Component with Oxytocin from Beef Glands. Paper electrophoresis on Whatman 3MM, 22 in long × 4 in wide. Mobility in mm indicates direction of movement. Paper chromatography on Whatman 1 in a descending manner.

Sample	Paper electrophoresis pH 3.5 (mm)	pH 6.5 (mm)	Paper chromatography Butanol-acetic acid-water (4:1:5) R_f
Oxytocin	−93	−105	.61
Human oxytocic component	−93*	−105	.62

* Tested for activity from unsprayed strip and found to have avian depressor activity.

The isolated fractions were characterized by various means. The peak containing vasopressor activity was assayed in both rat and bird. The ratio of activities was about 8 to 1, respectively, which is approximately the same as a sample of arginine vasopressin tested under identical conditions.

Technics of paper chromatography and paper electrophoresis were used to compare isolated samples with authentic samples of oxytocin, lysine and arginine vasopressin. In addition to detection of the components on paper with ninhydrin and bromphenol blue, the

TABLE III. Comparison of Human Pressor Component with Arginine and Lysine Vasopressin. Paper electrophoresis on Whatman 3MM, 22 in long × 4 in wide. Mobility in mm indicates the direction of movement. Paper chromatography on Whatman 1 paper in a descending manner.

Sample	Paper electrophoresis pH 3.5 (mm)	pH 5.6 (mm)	pH 6.5 (mm)	Paper chromatography (1)	(2)
Lysine vasopressin	−95	−137	−118	.08	.26
Arginine "	−93	−125†	−109†	.104†	.284†
Human pressor component	−92*	−128†	−108†	.102†	.295†

(1) Butanol/acetic acid/water—4:1:5. (2) Butanol/acetic acid/water/pyridine—30:6:24:20.

* Tested for activity on unsprayed strip and found to possess pressor activity.
† Positive test for arginine when sprayed with Sakaguchi reagent(9).

samples containing the vasopressin were also sprayed with the Sakaguchi reagent.

The results indicated that the sample of human oxytocin behaved in a manner identical to that of beef oxytocin after electrophoresis at pH 6.5 (Table II). In the case of the human vasopressin, the behavior on paper chromatography and paper electrophoresis was identical to that of arginine vasopressin (Table III). In addition the spots due to the vasopressin of the human gave a positive color with the Sakaguchi reagent.

Aliquots of each preparation were hydrolyzed in 6 *N* hydrochloric acid and the amino acids were identified by 2 dimensional paper chromatography, with butanol-acetic acid-water (4:1:5) for the first dimension and phenol-water (75:25) for the second dimension. All the amino acids found in oxytocin were present as well as traces of several others. The pattern obtained from the vasopressin hydrolysate showed the presence of only those amino acids found in arginine vasopressin. In addition, the basic residue from the vasopressin was also identified following paper electrophoresis of the hydrolysate (Table IV). At pH 5.6, in pyridine acetate buffer, a spot was obtained from human vasopressin hydrolysate agreeing in mobility with that of arginine and giving a positive test with the Sakaguchi reagent (Fig. 1).

TABLE IV. Paper Electrophoresis of Acid Hydrolysate of Pressor Component. Conditions: pH 5.6, pyridine acetate buffer, 400v, 4-5 ma, 5 hr; Whatman 3MM paper, 22 in long × 4 in wide. Mobility in mm indicates direction of movement.

Sample	Migration (mm)	Sakaguchi reagent
Lysine, 10 μg	−130	Negative
Arginine hydrochloride, 10 μg	−121	Positive
Human vasopressin hydrolysate	−19, −120, +88, +103	" 120 mm

It will be recalled that a large fraction of the activity remained in the supernatant solution after the proteins were salted-out with sodium chloride. It was necessary to examine this fraction and to determine the identity of the remaining active components. The proteins were removed by precipitation with a final concentration of 10% trichloracetic acid. The supernatant solution was added directly to a column of IRC-50. After removal of trichloracetic acid the components were separated by the gradient elution technic. Although the separated components were far from pure, the behavior of the active material on the column agreed with the results obtained with oxytocin and arginine vasopressin. Additional tests on the isolated fractions substantiated this point.

It is evident that the active components in an extract of posterior pituitary gland of the human can be accounted for by the presence of the known structures for oxytocin and arginine vasopressin. The hormones of human posterior pituitary agree in composition with hormones of beef rather than the hog. It is rather interesting that in the 3 cases so far examined, the oxytocin of the beef, hog and human are the same.

Summary. The oxytocic and pressor activities of the human posterior pituitary gland were separated chromatographically after preliminary purification. The behavior of purified components on ion exchange chromatography, paper chromatography and pa-

per electrophoresis indicated that they possessed the properties of oxytocin and arginine vasopressin. The amino acid composition of the oxytocin and vasopressin isolated from the human glands was determined. It is evident that the oxytocin and vasopressin found in the human pituitary are identified as oxytocin and arginine vasopressin.

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Effect of Cortisone and X-radiation on RNA and Glycogen Content of Rat Hepatocytes.* (24155)

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It was previously observed that DNA content of rat liver nuclei decreased both during cortisone administration and following x-radiation. In the former instance, the fall was progressive with continuing administration of the hormone; in the latter, there was a return to normal 5 days after x-radiation. Furthermore, daily cortisone administration to rats previously exposed to radiation caused a fall in DNA/nucleus and prevented the return to normal values at 5 days. A hypothesis was also presented to account partially for these observations. It was suggested that the apparent losses and increases in DNA/nucleus were due to depolymerization and repolymerization of the DNA(1). It appeared pertinent to study the effects of cortisone and/or x-radiation on the RNA and glycogen content of rat liver.

Materials and methods. Male rats of Wistar strain,†, weighing between 100-120 g were fed *ad libitum* on Lablox R diet.‡ Four groups of animals were studied: Group 1 received no treatment and served as control; Group 2 was injected with cortisone intramuscularly (25 mg/day) for one to 5 days and sacrificed after appropriate fast: Group 3 was exposed to x-radiation (1300 r) and killed from one to 5 days after x-radiation; Group 4 was cortisone-treated post-x-radiation; cortisone administration started within one hour after x-radiation and was given for one to 5 days. Details regarding cortisone dosage, frequency of administration, dosage and conditions of x-radiation and other precautionary steps were identical with those described previously(1). All animals were fasted 24 hours before removal of livers and were sacrificed between 9:00 and 10:00 a.m. to obviate diurnal variations in metabolism.

Cellular disruption and chemical analyses. At intervals after appropriate treatment, fasted animals were sacrificed and a 20% homogenate of liver pulp was prepared in slightly alkaline saline (containing 2 ml of 0.1 N NaOH/L of 0.85% NaCl W/V). Nucleic acids were extracted from a portion of the brei by procedure of Schneider(2) using hot trichloracetic acid (TCA), following removal of acid soluble materials with cold 5%

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‡ Allied Mills, Peoria, Ill.