FIG. 1. (A) Aorta of non-operated rabbit on high cholesterol diet; (B) aorta of sympathectomized rabbit on high cholesterol diet; (C & D) aortas of sympathectomized rabbits with aortic constriction on high cholesterol diet. (Arrow points to site of constriction.)

sult of decreased filtration of lipemic plasma through the intima.

The mechanism by which sympathectomy increases the severity of abdominal atherosclerosis in rabbits is not clear. Murphy(1) postulated that it is the combination of 2 factors, first, decrease in peripheral resistance and consequent increase in blood flow, and second, weakening of muscular wall of the artery due to paralysis of smooth muscle fibers. It is also possible that the metabolic transport capacity of the normal rabbit lumbar aorta is better able to handle the filtered substances than is the denervated vessel.

Summary and conclusions. Rabbits subjected to bilateral lumbar sympathectomy and fed a high cholesterol diet developed severe atherosclerosis in the lumbar aorta and iliac arteries. When an aortic constriction was concomitantly produced, atherogenesis distal to the constriction was diminished. This was accompanied by a decreased systolic and diastolic pressure, a diminished pulse pressure, and absence of visible aortic pulsation below the constriction.

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Effect of Bovine Fraction VI on Thyroid Function in the Rat. (24421)

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When normal human serum is labeled with tracer dose of I^{131} thyroxine and electrophoresis carried out on paper strips at pH 8.6 in a barbital buffer $\mu = 0.075$, radioactivity will be located in region of the α-globulins(1), provided the thyroxine level is not too high. Robbins, *et al.*(2) have demonstrated that this is due to the presence at pH 4.5, of a thyroxine-binding protein (TBP) with an isoelectric point close to pH 4. Freinkel, *et al.*(3) have demonstrated the presence of such a TBP in human serum fraction VI but confining their efforts to fraction IV-6 have isolated in very low yields a TBP whose binding capacity appeared to be easily lost(4). We have chosen to work with fraction VI since this fraction, according to the Cohn scheme of fractionation, is richest in glycoprotein.

Method. Bovine fraction VI (BFr VI) isolated according to an extension of Cohn method 10(5) by Pentex Corp. was labeled with I^{131} thyroxine, obtained chromatographically pure from Abbott Laboratories and electrophoresis carried out in duplicate on paper strips under conditions described above. The Spinco Model R paper electrophoresis apparatus was used. In the experiments recorded here, 20 λ of labeled protein solution

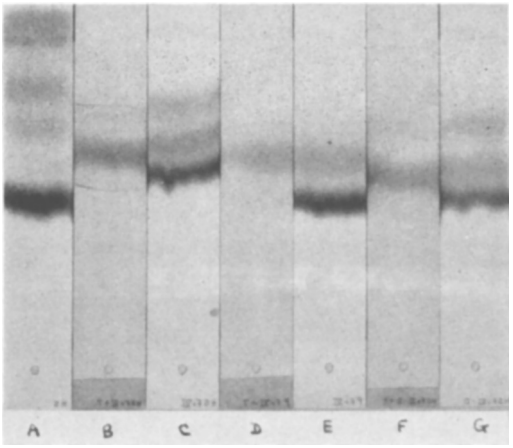
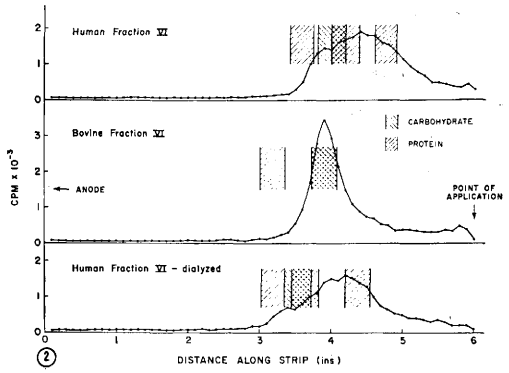


FIG. 1. Electrophoretic patterns of human and bovine fractions VI. A—normal human serum, bromphenol blue stain; B—human serum fraction VI, Köiw's stain; C—human serum fraction VI, bromphenol blue stain; D—bovine serum fraction VI, Köiw's stain; E—bovine serum fraction VI, bromphenol blue stain; F—human serum fraction VI-dialysed, Köiw's stain; G—human serum fraction VI-dialysed, bromphenol blue stain.

was applied to the paper, the concentration of protein being 2.6% for BFr VI and 2.8% for human fraction VI (HFr VI) dialyzed and undialyzed. In all these cases, 0.73 μc of I^{131} thyroxine was included in the 20 λ applied to the paper, corresponding to 5.2×10^{-2} γ of thyroxine. Exactly similar results have been obtained in which the protein concentration was 5.2%, thyroxine activity 0.69 μc corresponding to 2.40×10^{-2} γ thyroxine. Considering that only 10 g BFr VI can be obtained from 13 liters of plasma, these thyroxine levels are well below saturation for the TBP. One paper strip was then scanned for radioactivity and stained with bromphenol blue to indicate protein bands, and the duplicate strip was stained with Köiw's stain(6) to indicate carbohydrate bands. Fig. 1 and 2 show that under pH and ionic strength conditions used, 2 protein bands appeared, one of which represents a glycoprotein. Moreover, in this sample of bovine fraction VI, essentially all radioactivity was located in a narrow band coincidental with the glycoprotein band. This result has been reproduced many times with this same bovine fraction VI, and similar results were obtained with samples from other batches. HFr VI, also obtained from Pentex Corp., by method 10 of Cohn, examined electrophoretically under the same conditions always showed a less clear picture. Three protein bands were evident (Fig. 1, 2), one of which was characteristic of glycoprotein, while thyroxine seemed to spread itself out far more, as indicated by the radioactivity scan, than was the case of the BFr VI. The effect of BFr VI on thyroid function in the rat has been investigated. The properties studied were 1) 24-hour thyroid uptake of iodide ion, 2) 24-hour conversion ratio of inorganic to protein-bound iodine, and 3) body retention of iodine. Four- to 5-month-old female rats of Sprague-Dawley strain were given intravenously in the tail, various doses of BFr VI dissolved in normal saline solution. When multiple doses were given, these were at 24-hour intervals. Twenty-four hours prior to withdrawal of blood by cardiac puncture, they were injected with 5 μc of carrier-free I^{131} iodide, simultaneously with the last dose of Fr VI. After 4-6 ml of blood had been drawn, the rats were



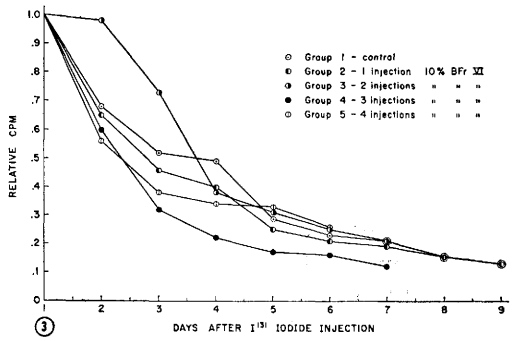


FIG. 2. Radioactivity scans of electrophoretic strips.

FIG. 3. Effect of bovine fraction VI on body retention of iodine in 5 groups of rats. The error (mean deviation from mean) in each point is, for the most part, $<10\%$ and $>20\%$.

TABLE I. Depression of Rat Thyroid Activity by Intravenous Injections of Bovine Fraction VI.

Treatment	Dose	No. of rats	C.R.	Thyroid, cpm × 10^{-3}	Thyroid count / Serum count
None	None	6	35.9 ± 2.7	295 ± 88	50.5
"	"	8	32.8 ± .7	146 ± 39	50.1
"	"	10	53.1 ± 5.0	164 ± 23	61.4
BSA*	1 × .4 ml 10%	4	37.4 ± 4.9	118 ± 20	76.0
"	4 × .5 20	7	41.3 ± 5.5	305 ± 55	55.6
BFr VI	1 × .5 20	4	26.1 ± 2.7	36.9 ± 12.6	13.9
"	4 × .5 20	13	16.1 ± 5.4	263 ± 44	41.8
None†	None	5	27.9 ± 5.5		
BSA†	1 × .2 ml 10%	10	26.2 ± 9.2		
BFr VI†	1 × .2 "	9	30.4 ± 6.7		
BSA‡	1 × .4 ml 10%	4	63.2 ± 5.3	78.5 ± 3.9	88.2
BFr VI§	1 × 1.0 "	5	14.6 ± 9.3	154 ± 20	10.8
"	1 × 1.0 "	4	33.1 ± 8.7	62.8 ± 15.3	12.1

4 × .5 ml 20% indicates 4 doses of .5 ml of 20% solution given at 24-hr intervals. Errors are mean deviations from mean.

* Some serums were pooled. † 7-wk-old, female Sprague-Dawley rats. ‡ 48-hr conversion ratio and thyroid count. § Results unreliable due to difficulty of inj. 1 ml into tail vein.

killed by overdose of ether, and their thyroid glands, together with a small section of trachea were removed and counted in a well-type scintillation counter. Serum was separated from coagulated blood after clot and cells had been centrifuged, and from 2-3 ml of serum were counted in the scintillation counter. From this counted volume of serum, the proteins were precipitated by addition of excess 10% trichloracetic acid, (TCA) centrifuged, and washed thoroughly with small volumes of 10% TCA, this procedure being repeated twice. The protein precipitate was then counted in the scintillation counter, the ratio of counts/minute of the protein to that of serum being the conversion ratio (C.R.). Two control groups of rats were treated similarly: the first group received only I^{131} iodide; the second crystalline bovine serum albumin (BSA) (Armour) in exactly similar doses to those receiving bovine fraction VI. The results are summarized in Table I. In body retention experiments, 5 groups of 5 rats were given increasing doses of bovine fraction VI intravenously in the tail, followed by a 5-μc tracer dose of I^{131} iodide. The whole rat was then counted on successive days in a large well-type scintillation counter, and the mean value for each group, relative to the count obtained one day after I^{131} iodide injection was taken as a measure of body retention of iodine. Group 1 received no bovine fraction VI and therefore acted as control; the remaining 4 groups received, respectively 1, 2, 3 and 4 injections of 0.4 ml of 10% bovine fraction VI, given at 24-hour intervals, the last dose given simultaneously with I^{131} iodide.

Results. The results are shown in Fig. 3. No significant differences were observed in renal clearance of I^{131} between treated and untreated rats.

Table I shows that the conversion ratio for rats treated with bovine serum albumin does not differ significantly from that of untreated controls, whereas a single dose of 0.5 ml 20% BFr VI resulted in a significant fall, and, 4 times this dose resulted in a marked drop in conversion ratio. Thyroid uptake measurements, as indicated by ratios of thyroid to serum counts, suggest that uptake is initially inhibited by comparatively small doses of BFr VI but unaffected by BSA. However the Table suggests the possibility that prolonged doses of BFr VI allow the thyroid gland to recover its capacity to trap iodide ion.

Conclusions. Bovine fraction VI has a component, possibly a glycoprotein, capable of binding thyroxine to the exclusion of other components: moreover, bovine fraction VI markedly inhibits conversion of inorganic iodide ion to organic protein bound iodine in the rat. This inhibition can be attributed nei-

ther to decreased body retention of iodine nor to foreign protein reaction, but seems to take place either at organification of the iodine stage or at subsequent stages that lead to secretion of thyroid hormone. It has been shown that thyroxine or desiccated thyroid gland(7) as well as triiodothyronine(8) given either orally or intravenously produce an immediate depression in thyroidal uptake of iodide ion but leaves the level of protein-bound iodine (PBI) unchanged. The depressive effect lasts beyond removal of exogenous thyroxine. It does not therefore seem probable that the depressed conversion ratio is a result of thyroxine or triiodothyronine bound to the glycoprotein component of fraction VI.* We are currently carrying out fractionation procedures to separate or at least concentrate this factor.

Summary. Serum fraction VI from a bovine source showed on paper electrophoretic separation the presence of a glycoprotein band capable of binding I^{131} labeled thyroxine to the exclusion of other proteins present. On being given intravenously in the tail of rats, bovine fraction VI gave a marked depression in conversion ratio of inorganic to protein-bound iodine. This effect was shown to be due neither to a foreign protein reaction nor to a decreased body retention of iodine. The uptake of iodide ion by the thyroid gland did not appear to be the cause of this depressed conversion ratio.

* In fact, we have examined samples of BFrVI for iodine content and have found the complete absence of iodine. These results have been presented for publication in this Journal.

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Thyroid Depressant Activity in Bovine Fraction VI. (24422)

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We reported(1) the presence of a thyroxine-binding protein (TBP) in bovine fraction VI (BFrVI) which was identified as a glycoprotein. It was also reported that BFrVI has a depressant effect on thyroid activity, as measured by the conversion ratio, when given intravenously to the rat. It was suggested that this effect might be attributable to thyroxine(2) accumulated by the TBP. We have since obtained many samples of BFrVI which the present paper shows have markedly higher thyroid depressant activity than those previously described, and have demonstrated that the activity is not due to presence of thyroxine nor to versene or heavy metals which may be present as impurities. The method for assaying the BFrVI for thyroid activity has been described(1). Sprague-Dawley female rats, weighing 250 to 300 g each, were used unless otherwise stated. Table I gives evidence of the markedly increased thyroid depressant activity of 2 samples of the same lot of BFrVI,* a single dose of 0.3 ml of a 20% fraction VI solution giving a conversion ratio of 16.8 compared with a value of 16.1 $\pm$ 5.4 previously obtained with 4 doses of 0.5 ml of a 20% solution(1). *Determination of*

* Obtained from Pentex Corp.