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UNIVERSITY OF LOUISVILLE

THE EFFECT OF ALCOHOL ON THE BODIES OF GUINEA
PIGS WITH SPECIAL REFERENCE TO THE THYROID GLAND. ✓

A Dissertation

Submitted to the Faculty

Of the Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

Of Master of Arts
Science

Department of Biology

by

A. F. STONER ✓

1932

DEDICATION

My highest appreciation and deepest
gratitude are due Dr. Austin Ralph
Middleton of the Department of Biology
of the University of Louisville for
his sympathetic direction and constructive
criticism of this study.

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INTRODUCTION AND HISTORY

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The recent discoveries in the field of endocrinology has led to extensive study in the pharmacological effects of various drugs on the glands of internal secretion.

The thyroid gland in particular has been found to change its structure and appearance when certain drugs are administered to experimental animals.

As far as I have been able to observe, there has been no investigation of a microscopic nature of the effect of alcohol upon the thyroid gland. It was, therefore, decided to study this problem and to make some general observations of the effect of alcohol upon the growth and pregnancy of guinea pigs.

The earliest description of the minute structure of the normal thyroid gland is that of Langendorff (10). Jackson (9) citing Langendorff (10) describes two kinds of cells in the thyroid gland. He gives them the names of principal cells and colloid cells. He found the principal cells to constitute the main mass of epithelial tissue. They are columnar cells and have reticular cytoplasm in which there are granular nodal points, and a round nucleus at the basal end of each cell. He found the colloid cells differed from the principal cells by the transparent appearance of the cytoplasm. He regards the colloid cell as the cells engaged in secreting colloid, but did not commit himself definitely to the opinion that they might return to the state of the principal cell.

Citing Rabelle and Da Costa (15) Jackson (9) showed that the thyroids of normal rabbits are variable

in structure with the occurrence of epithelial desquamation, resulting in the obliteration of the follicle. It was found that by injecting rabbits with thyroid proteid extracts this degeneration might be hastened.

Marine and Lenhard (13), by extensive investigation and experimentation on human and animal (dog, sheep, ox and pig) thyroids, found three types of follicular epithelial cells:

1. The normal resting cells of the low cuboidal type;
2. The hyperactive cells, becoming hyperplastic and columnar with colloid disappearing;
3. Cells of the colloid stage (stage of recovery) in which condition the cells returns to the first type, with abundant colloid material. If the hyperactive stage continues they claim that the cell dies of exhaustion, becoming degenerated and desquamated. The colloid in the cells of long continued hyperactivity will have completely disappeared.

Crowdy (3), working with thyroids of guinea pigs, found that in the majority of cases reticular cytoplasm lies between the basally located nucleus and the surface of the cell exposed to the colloid-filled lumen of the tubule. He thinks that the position of this reticular cytoplasm is a clue to the direction of secretion. The small percentages of reversals in the position of reticular cytoplasm in the epithelial thyroid cells of guinea pigs suggests to him that the balance

in production of secretion is in favor of storage rather than immediate discharge.

Jackson (9) found that in albino rats there occurs in most normal animals a spontaneous degeneration of the secreting colloid cells and colloid substances of the gland.

1. That there are functional changes (a change from the resting condition to the hyper-active condition, and then to the colloid condition);
2. That changes in the follicular epithelial cells can be produced by special diet (meats and by the administration of iodine);
3. That toxic substances (proteid extracts) may cause the epithelial cells to change from their normal structure;
4. That with age there is a tendency for follicular cells to become flattened (senile atrophic changes).

Bensley (2) shows the origin of the intra-follicular colloid of the thyroid. He shows that the substance is a true product of the epithelial cells inasmuch as globules of similarly staining substances were found in these cells. He explains the process of secretion as follows:

1. The follicular epithelial cells of the thyroid gland produce the colloid, since one can see in them little globules, having

- the same microchemical reaction as colloid;
2. The follicular cavity increases in size, this increase being caused by an increase in the cavity and also by an increase in the amount of colloid material in it;
 3. After the vessel is filled, its contents are discharged into the nearest lymphatic vessel;
 4. At last the collapsed vessel disposes itself in the form of little acini which repeat the process.

Ma (12) shows that the cells of the thyroid gland in the adult white rat pass through a secretory cycle, the chief feature of which is the formation of mitochondria which later dissolves in cytoplasmic vacuoles to form colloid.

The pharmacological effect of drugs and other materials on the thyroid gland was first studied by Wiss (22) in 1899. He studied the effect on the thyroid gland produced by poisoning with pilocarpine. He found that glands of dogs and cats treated with pilocarpine were larger and the nuclei of the follicular epithelial cells were less apparent. The free ends of the cells were prolonged into processes which were continuous with the colloid mass. He noted an abundant (abnormal) blood supply.

Galeotti (6) studied the thyroid gland of the turtle, emysa europaea, under normal conditions and after injection of various products of metabolism. He describes

two kinds of secretions from the colloid cells:

1. Fuscinophilic granules of nuclear origin;
2. Droplets of colloid.

Hunt and Seidell (7) in experiments on the effect of thyroid extract upon the resistance of animals to certain poisons found that the physiological activity of the thyroid depends upon its iodine content.

Swingle (20) experimented with thyroid/^{fed}Rana
Pipiens and found that experimental animals showed great bodily changes. Within a few days entire organs were transformed from the larval condition to that characteristic of the adult, and yet in the midst of such somatic formations, the gonads and germ cells remained unchanged.

Speidel (17) fed desiccated sheep's thyroid extract to Rana Calamata and found that after seven days of thyroid feeding the amphibian thymus contained:

1. More lymphocytes;
2. That the size of its blood sinuses were decreased;
3. That its lymphocytes exhibited increased migratory activity.

He found that in an animal killed thirty-three days after the first administration of thyroid extract, the blood sinuses of the thymus contained many young erythrocytes. He concluded that this was due to the hyperactivity of the thyroid caused by the thyroid feeding, and that the young erythrocytes had had their origin from the lymphocytes of the thymus.

Bensley (2) found that iodine administered daily to specimens, having hyperplastic thyroid glands, produces a slow accumulation in the follicular cells of the colloid, usually a single "drop" to a cell, and finally discharges these "drops" into lumens newly formed by the displacement of desquamated cells.

Watson (21) and Bensley (2) have noted the effect of meat diets on the thyroid gland. Watson (21) studied the effect of a meat diet upon the thyroid gland in Albino rats. The experiment was conducted for four months. Ten of the twelve treated animals showed marked changes in the thyroid, including congestion, epithelial congestion, epithelial proliferation of the follicle, and decrease in the amount of colloid in the follicle. In a later work he found that in young rats fed on an oatmeal diet for several weeks, a marked enlargement of the thyroid occurred. In some cases the epithelial cells were swollen and detached.

Bensley (2) found that by feeding specimens on a meat diet he could increase the number of vacuoles in the thyroid cells. In some of the animals thus fed, the vacuoles comprised fully half of the cell content. By fixing the glands of the normal and treated animals in Zenker's fluid and by staining with basalin-phospho-tungstic-acid solution, he found a new secretion antecedent. The substance was in the form of vacuoles. It was a dilute solution similar in its properties to the colloid of the follicular lumen, differing only from the latter in density. He states that the nor-

mal secreting cells of the thyroid secrete directly into the blood or lymph vascular channels, but if adjustment is disturbed so that the rate of secretion is in excess to the bodily needs, the indirect mode of secretion is brought about, and the products of the secretion are stored in the interfollicular cavity. According to this conception, the colloid in the thyroid vessel is no measure of the activity of the gland at the time of observation. Also, the lack of colloid in the gland does not necessarily mean that there has been deficiency in the activity of the gland, though it does mean that there has been a deficiency secretion or else the gland has never risen above the level of a secretory rate needed for direct export.

There have been many experiments to show the effect of alcohol upon the organs and organ systems of experimental animals. Chief among the experimenters are: Arlitt and Wells (1), Stockard and Papanicolaou (19), Pearl (14), Richter (16) and Sellmann (18).

Arlitt and Wells (1) reported that the administration of alcohol in the food of male white rats for two or three months resulted, in most cases, in the appearance of morphological degenerate changes. For instance, there occurred alterations of the testis, although other organs were apparently unchanged. They stated that alcohol affects the steps in spermatogenesis, and that before sterility and complete aspermia, the animal produces spermatozoa characterized by all degrees of abnormality.

Steckard and Papanicolaou (19) found that by treating guinea pigs with fumes of alcohol for one to two hours daily, there was no change in the external appearance of the liver, kidney, lungs, testis or ovary; but the germplasm of such individuals was altered:

1. The offspring are smaller and reduced in number;
2. There is frequent absorption of embryos in the alcoholized mothers. The latter condition is a rare occurrence in normal mothers.

Pearl (14) gave Barred Plymouth Rock chickens thirty-five to forty c.c. of either Methyl or Ethyl alcohol daily in the form of fumes for a period of fifteen months and noted that the mortality of the treated birds was much smaller than among their control brothers and sisters. At the beginning of the treatment body weight of the treated birds increased; after the initial rise, there was a distinct fall in weight which lasted for three months, to be followed by another rise. At the conclusion of the experiment the body weights of treated birds were greater than those of the normal ones. A large percentage of eggs from alcoholized hens were found to be unfertile.

Richter (16) found that alcoholized rats (given alcohol daily in water, eight to sixteen per cent of the volume of water) ate less than control rats. The animals weighed the same at maturity and seemed to grow as rapidly.

Sellmann (18) found that the continuous consumption by rats of Ethyl alcohol, in doses 2.7 to 9.4 c.c. per kilogram of weight per day, interferes considerably with their growth, and diminishes the consumption of food. The mortality rate, even after treatment for months, was not large.

The reasons for choosing alcohol as the treating agent were:

1. That considerable knowledge exists concerning its pharmacological action on certain animal tissues;
2. It is known to be an organic substance which might produce effects on the thyroid gland;
3. Because of Lorand's (11) hypothesis that drunkenness is inherited due to the modification of the thyroid gland, which, he said, in turn is inherited.

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II

GROSS EXPERIMENTAL STUDY ON THE GUINEA PIGS

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(A) - MATERIALS AND METHODS FOR CARE OF GUINEA PIGS

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A group of sixteen guinea pigs, eight males and eight females, was obtained from a reliable breeder, Mr. Lottie Mahand, in September, 1930. The animals were from three litters.

1. Litter one contained the following animals:

A1♀, A2♀, A3♀, and A4♂.

2. Litter two contained:

B1♂, B2♀, B3♂, and B4♂.

3. Litter three contained: C1♂, C2♀, C3♀, and C4♂.

D1♂ and D2♂ were the offspring of A2♀ and A4♂ before either parent was treated with alcohol. D3♀ and D4♀ were the offspring of A1♀ and A4♂ before either parent was treated. The average age of the animals when treatment began was eight months. The animals were all strong and vigorous in appearance. On October 1, 1930, animals A3♀, B2♀, C2♀, A4♂, B1♂, C4♂, were selected for alcoholic treatment. The choice was entirely at random, there was no evidence of superiority or inferiority in the animals selected for treatment against those selected as controls. The animals retained as controls were: A1♀, A2♀, C3♀, B3♂, C1♂, B4♂; on March 26, 1931, D1♂, D2♂, D3♀, D4♀, were introduced into the experiment. D4♀ and D2♂ were treated with alcohol while D1♂ and D3♀ were retained as controls.

Throughout the experiment Ethyl alcohol was administered to the guinea pigs orally by means of a

pipette. 5 c.c. of either 19%, 27% or 33% concentrations by volume of Ethyl alcohol was used. The animal was held in position on the operator's lap by his left hand and the pipette was manipulated with the right hand. With a short period of conditioning for the animal and improvement of technique on the part of the operator, the animals were made to consume the alcohol.

At the beginning of the experiment, 5 c.c. of 19% concentration of Ethyl alcohol was given daily. This concentration was used only fifteen days as it soon became evident that the desired intoxication was not being produced. On October 15, 1930, the concentration of the alcohol was increased to 27%. This dosage was not yet adequate for the desired intoxication and so on November 6, 1930, the concentration was increased to 33%. From this date throughout the experiment the dosage remained the same.

All of the guinea pigs, both experimental and control, were kept in the same type of cage. The cages were built on the individual cage plan. One hatch was constructed which contained forty compartments. Each compartment was two and one-half feet long by one foot wide. Each cage was large enough to accommodate one animal, or one female and her young. The accommodations for both the experimental animals and control animals were the same. The cages were thoroughly cleaned, the floor sprinkled with sawdust, and in winter hay put in daily. Each animal, control and experimental received approximately 300 grams

of feed per day. The feed consisted of two kinds:

1. Dry food;
2. Green food.

The dry feed contained the following constituents:

1. Relled Oats - 45%
2. Bran - 35%
3. Oil Meal - 5%
4. Tankage - 5%
5. Molasses Feed- 10%

The green food consisted of green vegetables obtained as refuse from wholesale houses and dandelions. It was also necessary for their perfect health that the guinea pigs be given fresh water daily and during the summer it was found advisable to change the water twice each day.

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(B) - MATINGS OF THE GUINEA PIGS

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In mating the animals, a male and female were placed in a compartment (breeding pen) during the female's oestral period. Matings of the animals were made after experimental animals had been treated with alcohol for fifteen days. After the male and female were together for one month, an examination of the female was made. It consisted of feeling the horns of the uterus to determine the number of embryos. The following matings were made:

A4♂	alc.	X	A3♀	alc.
A4♂	alc.	X	A1♀	
B2♀	alc.	X	B3♂	
C1♂		X	C3♀	
C4♂	alc.	X	C2♀	alc.
B4♂	alc.	X	C2♀	alc.
B4♂	alc.	X	A2♀	
D3♀		X	D1♂	
D4♀			D2♂	alc.

Careful observations were made daily to observe the condition of the pregnant animals. Not a single abortion or absorption of embryos was found. However, Stockard and Papanicolaou (19) reported that absorption of embryos by alcoholized mothers was frequent.

The results of the above matings were:

A4♂	alc.	X	A3♀	alc.	3 normal young
A4♂	alc.	X	A1♀		3 normal young
B2♀	alc.	X	B3♂		3 normal young
C1♂		X	C3♀		3 normal young

C4 ♂	X	C2 ♀	2 normal young
B4 ♂	X	A2 ♀	3 normal young
D4 ♀ alc.	X	D2 ♂ alc.	2 normal young
D6 ♀	X	D1 ♂	2 normal young

The average weight of the young at birth from matings of normal parents was 59 grams, from matings of alcoholized parents 59 grams, and from matings of one normal parent and one alcoholized parent 58.5 grams.

Figure 1 shows a normal female (D3) and her offspring from a mating with a normal male (D1). (Photograph was taken when the young were ten days' old.)

Figure II shows an alcoholized female (D4) and her offspring from a mating with an alcoholized male (D2). (Photograph was taken when the young were ten days' old.)

Figure III shows a normal female (D4) and her offspring from a mating with an alcoholized male (D2). (Photograph was taken when the young were ten days' old.)

Figure IV shows an alcoholized female (B2) and her offspring from a mating with a normal male (B3). (Photograph was taken when the young were ten days' old.)

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(c) - GENERAL EFFECTS OF ALCOHOL-
UPON THE BODIES OF GUINEA PIGS

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The general effects of 5 c.c. of 35% Ethyl alcohol on the guinea pigs were like those of general intoxication:

1. A lack of muscular coordination;
2. Slight seizures of sneezing and coughing;
3. Watery discharges from the mouth and nose during the first twenty days of treatment;
4. A glassy condition of the eyes;
5. Defects in the reactions. When a finger is jabbed at normal animals they retreat, but when jabbed at treated animals they remain quite still.

In a study of this kind, a comparison of weights is important. Table I shows:

1. The initial weight of each animal;
2. The total initial weight of the alcoholic group and control group of animals.
3. The weight for each animal at twenty day intervals for 200 days;
4. The final weight of alcoholic and control animals;
5. The final weight of alcoholic and control groups after 200 days;
6. The percentage of gain or loss for each animal at the end of 200 days.

From this table it is shown that alcohol had no effect on weight and that weight cannot be used as a criterion for the general effect of alcohol on guinea pigs.

Table I shows: (1) the initial weight of each animal, (2) the total initial weight of the alcoholic and control groups, (3) the weight of each animal at 20 day periods for 200 days, (4) the final weight of alcoholic group and control group of animals, (5) and the percentage of gain or loss for each animal at the end of 200 days.

CONTROL GROUP													
No. of animal	Initial weight grams	Weight 20th day	Weight 40th day	Weight 60th day	Weight 80th day	Weight 100th day	Weight 120th day	Weight 140th day	Weight 160th day	Weight 180th day	Weight 200th day	% gain	% lost
A1 ♀	788	815	556	523	548	555	562	killed					28.7
A2 ♀	599	899	490	431	448	552	559	562	660	672	690	15.8	
C3 ♀	376	382	399	503	526	461	471	481	671	674	777	80.	
B3 ♂	368	372	491	506	539	541	549	552	690	700	705	91.6	
C4 ♂	362	371	444	463	472	481	501	512	654	660	663	83.1	
B4 ♂	373	382	436	502	510	532	549	552	628	632	640	71.5	
D1 ♂										685	699	2.	
D3 ♀										691	693	.2	
Totals	2866	2921	2826	2928	3043	3122	3191	2659	3303	4714	4757		
ALCOHOLIC GROUP													
A3 ♀	669	670	872	533	462	474	488	562	720	722	730	9.1	
B2 ♀	325	334	423	488	492	532	548	551	680	690	682	109.8	
C2 ♀	334	339	362	439	450	459	562	555	576	577	590	73.6	
A4 ♂	664	672	615	601	614	632	628	648	710	700	713	6.9	
B1 ♂	332	367	408	501	522	532	killed					60.2	
C4 ♂	380	392	490	453	460	492	494	482	610	621	635	70.8	
D4 ♂										685	692	11.02	
D2 ♂										690	691	.14	
Totals	2704	2774	3170	3024	3001	3121	2721	2798	3296	4685	4733		

III

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EXPERIMENTAL STUDY OF THE THYROID GLAND OF GUINEA PIGS

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(A) - MATERIALS AND METHODS FOR THYROID STUDY

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On January 29, 1930, the first alcoholic animal was killed and sections were made of the various glands (liver, kidney, adrenals, thyroid and spleen). When these tissues were excised it was noted that the thyroid showed an abnormal appearance. All other glands appeared normal. Because of the condition of the thyroid it was decided to limit the study to this structure. In all, sixteen glands (eight experimental and eight control) have been sectioned and studied. The animals from which the glands were taken varied in age from eleven to fourteen months.

The thyroid tissue was obtained immediately after the animals were killed. Most of the tissue was fixed in Bouin's fluid from twelve to twenty-four hours. In some cases Zenker's or Flemming's fluid was used. The best results were obtained when the glands were fixed in Bouin's solution.

The glands were imbedded in paraffin and cut at eight microns (occasionally twelve microns). In a majority of cases sections were taken from different parts of the gland as the thyroid shows variations in structure in its different regions.

All drawings were made with a Leitz 4 mm. objective with a 15X eye piece, giving about 700 diameters' magnification, with the aid of an Abbe Camera Lucida, at bar length 125 mm.

An ordinary Bausch and Lomb micrometer eye piece 7.5X was used in all measurements.

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(B) - THE NORMAL THYROID GLAND

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The thyroid gland in guinea pigs is butterfly-wing shaped. The two lobes lie on the lateral surfaces of the trachea and larynx. The lobes are joined by a narrow, almost invisible isthmus. The gland in all cases is located under and slightly lateral to the infrahyoid muscle. The weight at the time of autopsy ranges from .9 to 1.4 grams. The average weight from alcoholized animals was 1.02 grams and from control animals 1.03 grams.

The minute structure of the normal thyroid gland of guinea pigs is shown in figure five.

The follicles of the gland in normal animals are rounded or oval in shape and vary from 12 to 148 microns in diameter. The inner and outer cell walls are distinct. The inter-cellular boundaries between the flattened cells are less distinct, and sometimes no boundary at all can be made out. This may be due to the fact that the flattened cells stain deeply.

The cytoplasm of the follicular cells is filled with a medium sized granular substance. When the thyroid is fixed in Bouin's fixative and stained with Delafield's hematexylin and counter-stained with eosin, these granules are blue. When fixed in Zenker's fixative and stained in the same manner they are red. Occasionally, along these strands of granules there may be found a minute, clear, watery vacuole interposed between adjacent granules.

The cells present a fairly uniform appearance and there is nothing in the cytoplasm or nucleus to indicate any division into colloid and chief cells described by Langendorff (10).

The nuclei of the follicular epithelial cells are slightly ellipsoidal in form, their diameters range from 6 microns to 8 microns. The average is 6 microns. The nuclear membrane is distinct and stains slightly deeper than any other part of the nucleus. There are from two to three nucleoli and several smaller granules found in the nucleus. The nuclear sap, karyolymph, forms a pale nuclear background. Cells undergoing mitosis are occasionally seen.

The colloid shown in figure five is typical in form. Peripheral follicles are much larger, however, and in them much more colloid is present. In some peripheral follicles, colloid fills the whole of the follicular cavity. In the deeper follicles the colloid occupies only the center of the follicular cavity and does not make contact with the epithelial cells at any point. This retraction may or may not be an artifact due to shrinkage caused by fixing reagents. Vacuoles are not numerous in normal glands, and when they are found, are small in size, and are not absolutely clear. They stain very faintly with eosin. These vacuoles, when found, measure from three to five microns. They are spherical in shape and are near the surface of the colloid material. The position of the vacuoles leads to the assumption that this vacuolar material was probably secreted by the colloidal epithelial cells.

Interfollicular connective tissue is of the fibrous kind, and forms a delicate fibrous stroma. The

amount of connective tissue is small, but contains a rich supply of blood vessels.

In glands from control animals, there are frequently seen degenerated cells. These may occur in any part of the gland. In some cases a single colloidal epithelial cell may be degenerated, in others all the cells of a single follicle may be in this condition. These cells may remain in the follicular wall or they may be desquamated into the follicular cavity. In some cases in glands from control animals, the author has seen desquamated cells occupying the whole of the follicular cavity. The cytoplasm of degenerating cells loses its typical light granular structure and becomes vacuolated and reticular in appearance. In later stages of degeneration, the cytoplasm becomes a deep-stained eosinophilic mass. The nucleus appears structureless, but this may be due to its great affinity for stain.

Figure V. A camera lucida drawing of a cross section of a normal thyroid gland showing several follicles. The structure in the upper right is a vein with its red blood corpuscles. Beneath this is an artery. Inside the follicles (which are ring shaped cellular structures) is a mass of colloid material, shown in dark gray. The white spaces in the colloid material are vacuoles. The cells which make up the border of each follicle are follicular epithelial cells. The fibrous material between the follicles is connective tissue.

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(C) - THE ALCOHOLIZED THYROID GLAND

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The appearance of thyroid glands from animals subjected to 5 c.c. from 19% to 35% Ethyl alcohol for periods ranging from 100 to 220 days is shown in Figure VI. The follicles in general appear slightly larger than those from glands of normal animals. The minimum diameter of the follicles was 20 microns and the maximum was 200 microns. The minimum diameter of follicles in glands from control animals was 12 microns and the maximum 148 microns.

The length of the epithelial cells of the thyroid follicles of alcoholized animals ranges from 3 microns to 13 microns. Flattened epithelial cells are numerous. They occur bordering internal follicles as well as peripheral follicles. In glands of control animals flattened epithelial cells are only found bordering colloid in the peripheral region. The average size of colloid epithelial cells from glands of experimental animals is about 4 microns and from control animals 6 microns. Since the whole follicle is slightly above normal in size and the epithelial cells are slightly below normal in size, it follows that the increase in size of the follicle is due to the increase in the colloid content.

The cytoplasm of the follicular epithelial cells of alcoholized animals has undergone marked change. Results show that its amount has been slightly reduced. The slight decrease in size is probably due to the decrease in amount of cytoplasm.

The cytoplasm is greatly changed in structure. It is highly vacuolated and the normal indefinite reticulum of granules has entirely disappeared, though occasionally fragmentary vestiges may remain. The cytoplasm now presents a somewhat reticular appearance. The appearance may be caused by the presence of more vacuoles within the cytoplasm. The author has observed these vacuoles along the margin of ruptured follicular epithelial cells in contact with colloid material. He thinks that these vacuoles are excreted along with the colloid material into the follicular cavity. Some vacuoles are found in normal cytoplasm, but the number and type of vacuoles are different in cells of alcoholized animals. The coarse vacuolization in the cytoplasm of the thyroid epithelial cells of alcoholized animals was never seen in that of control animals.

The cytoplasm of the very flattened colloid-
al epithelial cells of alcoholized animals is much reduced in content. Here there is no reticular appearance, and vacuoles may not be present. In this case the cytoplasm presents a homogeneous appearance, and stains deeply when stained with Delafield's hematoxylin and counter-stained with eosin.

The nuclei in general seem somewhat more resistant to alcoholic treatment than does the cytoplasm. In form and size the nuclei are normal. They range in

diameter from 2 to 8 microns. The average diameter in both normal and alcoholized animals is 6 microns. No modification in the structure of the nucleus has been noted. No cases of mitotic or amitotic cell divisions were found in alcoholized animals.

The colloid in follicles of glands from alcoholic animals has undergone change. Here the large and small follicles are evenly distributed, but in the normal glands the very large follicles seem to be restricted to the periphery of the gland. As already mentioned the follicular cavities of alcoholized animals are larger than those of normal animals. The material within the cavity stains lighter with eosin than does normal colloid. The colloid in normal glands, in many cases, does not fill the whole of the follicular cavity, but in a large number of cases in glands from alcoholized animals the colloid material completely fills the cavity. There are very few vacuoles in the colloid material of normal animals, but vacuoles are numerous in colloid material of treated animals. As many as 16 vacuoles may be counted within one follicle. When present in normal animals, these vacuoles are restricted to the periphery of the colloid mass, but in experimental animals they are also found deep within that mass. The size of the vacuoles ranges from 2 to 20 microns in experimental animals. Since vacuoles are found bordering the follicular cavity, within the epithelial cells, and since it has been noted that the cell wall bordering the follicular cavity often disappears, it is thought

that this vacuolar material of the colloidal mass is a product of the follicular epithelium.

The interfollicular (interstitial epithelium) appears to have undergone somewhat the same change in alcoholized animals as the colloidal epithelial cells already described.

The connective tissue in the thyroid of animals subjected to alcohol appears almost normal in amount and structure. In a few cases it appeared that more connective tissue was present. The evidence, however, is not conclusive. If more connective tissue is present it may be due to an infiltration of a clear ground substance or to a change in position of the epithelium. This change in position might have been caused by an increased pressure of the larger cells in the abnormal glands. The blood vessels appear quite normal.

There are present in glands from alcoholized animals more of the degenerated cells which appeared in normal glands. The degenerated cells occur singly or involving a follicle or follicles. Desquamated cells within the follicular cavity are numerous. Whole follicles of desquamated cells, whose cytoplasm has completely disintegrated into deeply staining eosinophilic masses, are frequently to be seen. In such cells the nuclei appear only as dark masses, due to their affinity for the stain.

Figure VI. A camera lucida drawing of a cross section of a thyroid gland from an alcoholized guinea pig showing a follicle and related structures. The structure in the upper right is an artery. To the left of this is a vein with its red blood corpuscles. In the center is a very large follicle. The dark mass within the follicle is colloid material. The white spaces in the colloid material are vacuoles. The cells which form the walls of the follicle are colloid epithelial cells. The fibrous material outside the follicle is connective tissue. At the right center are several degenerated and desquamated epithelial cells. At the upper left are four fat cells.

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IV

DISCUSSION WITH REFERENCE TO CITED LITERATURE

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While Langendorff (10) Marine and Lenhart (13), Jackson (9), and Ma (12) maintain that the thyroid epithelial cells of animals (dog, sheep, ox, pig and rat) pass through a secretory cycle in which the cells present various modifications from exhaustion to regeneration, the author finds, however, that the epithelial cells in normal thyroid glands of guinea pigs at the age of 440 to 550 days, present a fairly uniform appearance. When fixed in Bouin's or Zenker's fixative and stained with Delafield's hematoxylin and counterstained with eosin, the staining is uniform in all of the epithelial cells. There is little variation in size of these cells. The uniformity in appearance of the epithelial cells observed may be due to the type of stain employed, or to the assumption that the epithelial cells may not pass through a secretory cycle, but that the secretory action is a continuous process.

The evidence of this experiment substantiates the experiments of Crowdy (3) and Bensley (2), in which the origin of colloid material was found to be from epithelial cells. Observations from slides were made in which the epithelial cell wall bordering the follicular cavity was ruptured, and droplets of colloid and vascular material were found protruding into the cavity.

Wiss (22), who poisoned dogs and cats with pilocarpine, found that the blood supply of the thyroids from treated animals showed an abundant (abnormal) blood supply. In this experiment special attention was given observations concerning the blood supply of both normal

and alcoholized glands of guinea pigs. The vessels and blood cells from the alcoholized glands present the same appearance as those from the normal gland (Figure V and VI). There was no apparent increase in the count of the blood cells.

The change produced in the thyroid gland of opossums by the feeding of a meat diet as described by Bensley (2) is somewhat similar to that caused in the thyroid gland of guinea pigs by the daily administration of 5 c.c. of 19% to 35% by volume of Ethyl alcohol. In both cases:

1. The colloid material was increased;
2. The number of vacuoles were increased.

The results of the experiment do not agree with Bensley, however, in his assumption that the thyroid gland normally secretes directly into the blood or lymph stream, and that the indirect mode is only brought about when the rate of secretion is to the excess of bodily needs. The indirect method of secretion was the only method of secretion in this experiment. Observations of slides in both normal and alcoholized glands were observed in which the epithelial cell wall bordering the follicular cavity was ruptured and droplets and colloid and vacular material were found protruding into the follicular cavity.

No great, detailed histological study was made of the gonads, but at least no cases of sterility were noted. Arlitt and Wells (1) report, however, that

by the administration of Ethyl alcohol in the feed of male white rats for two or three months, that there often occurred complete sterility. In this experiment, slides of both the testis and ovary from alcoholized animals appeared normal.

The findings of this experiment do not agree with the findings of Stockard and Papanicolaou (19), that the offspring of alcoholized guinea pigs are smaller and reduced in number. It was found that:

1. The average number of offspring from alcoholized parents were the same as that from control parents;
2. The average weight of the young from alcoholized parent was 59 grams, which is the same as the average weight of the young from normal parents.

Neither were there found any absorptions or abortions of embryos in alcoholized mothers. This was a frequent occurrence in the experiment of Stockard and Papanicolaou.

Although Sellmann (18) found that the continuous consumption of Ethyl alcohol by rats interfered with their growth, it was not found to be the case with guinea pigs, as is shown in table one of this experiment. The table shows that alcohol has no effect on increase in weight and that weight cannot be used as a criterion for the general effects of alcohol on guinea pigs.

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V

SUMMARY

of

THE EFFECTS OF ALCOHOL ON THE THYROID GLAND
OF GUINEA PIGS

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1. The purpose of the study was to observe the general effect of alcohol on the body of the common guinea pig and to determine its effect upon the histological structure of the thyroid gland.

2. The daily administration of 5 c.c. of 35% by volume of Ethyl alcohol was found to produce the desired intoxication on the experimental animals. This treatment was used on eight animals for 200 days.

3. The general effects of 5 c.c. of 35% Ethyl alcohol upon the treated animals were:

1. A lack of muscular coordination;
2. Slight seizures of sneezing and coughing;
3. Watery discharges from the mouth and nose during the first 20 days of treatment;
4. A glassy condition of the eyes;
5. Defects in reactions.

4. The thyroid glands from both experimental and control animals were obtained at autopsy held immediately after the animals were killed. Sections were made, and the sections from experimental animals were compared with these from control animals:

1. The follicles of the thyroid gland from experimental animals are slightly larger than those from control animals;

2. The follicular epithelial cells are slightly smaller;
3. The cytoplasm of these follicular cells has changed from a granular appearance to a highly vacuolated condition. The quantity of cytoplasm has been slightly reduced. The nuclei remain unchanged;
4. The volume of colloid material is increased and it contains more vacuoles. It stains lighter, and in a majority of cases completely fills the follicular cavity;
5. The interstitial connective tissue is typical in form but greater in quantity;
6. Desquamated and degenerated cells appear more often in glands from alcoholized animals.

5. This experiment is the beginning of a series of experiments to determine the effect of alcohol on the thyroid gland of guinea pigs. Future papers will deal with the effect of alcohol on the filial offspring of parent animals.

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