A CHEMICAL STUDY OF THE SECRETION OF THE ANAL GLANDS OF MEPHITIS MEPHITICA (COMMON SKUNK), WITH REMARKS ON THE PHYSIOLOGICAL PROPERTIES OF THIS SECRETION.

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The subfamily *Mephitinæ*, to which the skunk belongs, is represented in the United States by three genera: *Spilogale*, *Conepatus*, and *Mephitis*. *Mephitis* includes all of the large common skunks of the United States except the so-called "badger" skunks along our Southern boundary, which belong to the genus *Conepatus*. Three species are referred to *Mephitis*: *M. macrura*, *M. putorius*, and *M. mephitica*. The latter species is found from Hudson's Bay to Guatemala, but is more frequently met with in the Northern and Eastern States.

Before considering the secretion itself it will be well to describe briefly the anal glands or pouches, common not only to the subfamily *Mephitinæ*, but also to the family *Mustilidæ*. The two pouches in which is secreted the fluid emitting the offensive odour that has made the skunk notorious are situated on either side of the rectum close to the anus. They are oval in shape, the longer diameter from an inch to an inch and a half, surrounded by a muscular envelope and opening into the rectum just inside the sphincter by single ducts which terminate at the top of prominent nipplelike papillæ. The papillæ are situated at the bottom of recesses, and when not protruded are nearly concealed from view by folds of the mucous membrane. By gentle pressure the fluid in the sac may be forced out through the duct in a very fine stream.

There is still prevalent an erroneous idea that the secretion is identical with the urine or is in some way connected with it. That

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there is no connection whatever between the sacs and the genito-urinary passages has been shown by Wyman * and Chatin.† The latter especially has made a thorough anatomical study of the anal glands of *Conepatus mapurito*. His observations may be summed up as follows: Below the muscular layer—that is to say, within the general muscular envelope—is found the follicular or glandular portion proper of the organ; it is not regularly distributed around the central reservoir, as in most carnivora, but occupies only a limited portion of the surface of the receptacle. The follicles are rather large and of a reddish-brown colour; their numerous well-developed *culs-de-sac* measure on an average 0·55 millimetre in diameter, and are variously rounded, ovoidal, club-shaped, etc. The reservoir is lined with a comparatively thick whitish tunic composed of dense laminated tissue and elastic fibres.

Dr. L. F. Barker, Associate in Anatomy in the Johns Hopkins University, has kindly furnished me with the following analysis of a series of sections from tissues obtained from the common skunk, which were prepared for me in the Pathological Laboratory by Dr. George W. Dobbin:

"The sections go through the wall of the sac in different parts, and have been stained in hæmatoxylin and eosin. The wall consists of an internal mucous membrane, a submucous coat, a muscular coat of striped fibres, and an external fibrous coat. The masses of glands are situated in the submucosa. Leading from the reservoir to the outside is a duct lined by stratified epithelium which opens at the apex of the papilla or nipple.

"*Tunica Externa.*—This is thin, consisting of fibrous tissue poor in cells. A few fat-cells are present in places, and large veins are met with in transverse and longitudinal section.

"*Tunica Muscularis.*—The muscular coat varies considerably in thickness, being about four millimetres in its thickest part and one millimetre in its thinnest part. It consists of bundles of long striated muscle fibres, which in places run all in one direction; at other parts of

* *Proc. Boston Soc. of Nat. Hist.*, 1844, i, p. 110.

† *Ann. des Sciences nat.* [5], 1874, xix, p. 100.

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the wall the fibres of the outer part run at right angles to those of the inner. The individual muscle fibres are of large size, are exquisitely cross-striated, and show spindle-shaped nuclei in the periphery of the fibre quite like those of human muscle. The internal perimysium carrying blood vessels is everywhere delicate.

"*Tela Submucosa.*—This consists of a rather loose meshwork of fibrous tissue intervening between the mucous membrane and the muscle. In it are situated the masses of glands. These are not evenly distributed throughout the wall of the sac, but occur in masses in various parts, being particularly abundant at the fundus of the reservoir. In the submucosa, arteries of medium size and larger veins are situated; and near the glands, between them and the muscle, bundles of medullated nerve fibres of considerable size are to be made out.

"The masses of glands themselves show gland tubes cut in various directions transversely, longitudinally, and obliquely. They appear to be coil glands, although they may branch. The amount of connective tissue between the coils is insignificant, there being just enough to carry a rich supply of blood capillaries. Sections of the glands show the tubules to be lined by cubical epithelium; the nuclei of the cells are situated rather nearer the proximal than the distal ends of the cells. They are round, or slightly oval, and stain intensely in hæmatoxylin. The distal portion of the protoplasm stains rather more feebly than the proximal portion, the latter taking a deeper tint in hæmatoxylin and tending to be slightly granular. The lumen of the tubule varies in different parts. In places it is wide, in other places reduced to a narrow slit. Between the proximal ends of the epithelial cells and the basement membrane long, spindle-shaped nuclei can be seen whose long axis is, as a rule, parallel to the course of the gland tubule. These are evidently the nuclei of smooth muscle fibres. The basement membrane itself appears delicate and homogeneous. Here and there within a mass of tubules an artery or vein of considerable size may be met with.

"*Tunica Mucosa.*—This consists of an epithelial covering and a tunica propria. The epithelial covering consists of several layers of

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epithelial cells, the deepest layers tending to be cubical, the more distant layers to be polyhedral in shape. As a rule, the layer is from four to five cells deep. All of the cells distal to the proximal row show a network within the cell protoplasm, the meshes of which are filled up by clear hyaline droplets. The appearances in these cells resemble very closely those to be seen in the cells of sebaceous glands.

"The superficial cells of the epithelial layer may be nucleated or non-nucleated, and in this layer the protoplasm contains large numbers of irregular masses and particles which stain deeply in hæmatoxylin, but metachromatically, taking a reddish-brown tint. These particles resemble closely those seen in the granular cells at the periphery of the stratum germinativum (Malpighii) in human skin (kerato-hyalin—Waldeyer). In places on the surface or desquamated from it can be seen a layer of cells which hang together, which are almost entirely devoid of chromatic substance and look like pale vesicles united edge to edge.

"The tunica propria of the mucous membrane is a little less than 1 millimetre in thickness, and consists of bundles of white fibres which for the most part run parallel to one another, taking a somewhat wavy course. Nuclei of connective-tissue corpuscles are visible between the bundles, and small blood vessels are also present in this layer. There are no distinct papillæ to be made out in the wall of the reservoir, although they are present in the duct leading from the reservoir through the papilla to its apex.

"*The Papilla or Nipple.*—In transverse section this is seen to be lined by stratified epithelium resting upon a distinctly papillated tunica propria. The papilla is rich in voluntary muscle fibres, the mucous membrane lining it being surrounded by two rows of bundles of fibres which run parallel to the long axis of the duct. External to these is a tolerably thick layer of muscle fibres which run for the most part circularly, although some of them run obliquely. Nowhere in the papilla or in the reservoir can smooth muscle be made out except, as before described, in the structure of the glands themselves. Outside the voluntary muscle of the papilla is a firm fibrous

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coat. In the connective tissue of the papilla and here and there among the epithelial cells branched pigment cells are visible.

"The histologist must be struck by the resemblance of the structure of the glands in the wall of the reservoir to that of the ordinary sudoriferous glands of the skin. Indeed, it is not inconceivable that these glands of the skunk may represent curiously modified sweat glands. As to the nature of the reservoir with its curious epithelial lining, it is difficult to decide. If the glands in the wall of the reservoir of the skunk should turn out to be homologous with sweat glands, the relation of the reservoir to sweat pores will have to be considered. In this connection, the curious character of the epithelium cells lining the reservoir, reminding one somewhat of the structure seen in sebaceous glands, must not be forgotten; and any attempt to explain the origin and the character of the secretion should consider not only the products of the glands in the submucosa, but also the part, if any, played in its formation by the epithelium of the reservoir itself."

The exceedingly disagreeable odour possessed by the secretion of the *Mephitinæ*,* and the difficulty attending its collection in sufficient quantity for an exhaustive study have deterred investigators in the past from studying this most singular and interesting fluid.

If one were bold enough to start the investigation, objections were invariably raised, and thus all further research was stopped. At least this was Loew's† experience, who says: "On an expedition through Texas in 1872 I had frequent opportunity to collect a sufficient quantity of this secretion to establish its chemical constitution, but all my companions protested against it, declaring the odour which clung to me to be unbearable. On my return to New York city I started a few chemical tests, with the little I had collected, when the whole college rose in revolt, shouting, 'A skunk, a skunk

* I am informed by Dr. C. Hart Merriam, Chief of the Division of Mammalogy and Ornithology, Washington, D. C., that the secretion of some other members of the family *Mustilidæ* is, if possible, more offensive than that of the skunk. The secretion of the mink is not so diffusible or penetrating, but it is more nauseating.

† *Aerztliches Intelligenzblatt von München*, June 10, 1879, p. 252.

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is here!' I had to abandon the investigation." * This, no doubt, has been the experience of others who have attempted to study either the secretion or the glands. I have been more fortunate than my predecessors in being surrounded by those who, for the cause of science, would endure even the odour of a skunk in close proximity. Had it not been for this forbearance on their part, I should undoubtedly have suffered the fate of others.

Lassaigne,† Swarts ‡ (in Wöhler's Laboratory), and Loew # have all made the secretion of the skunk the subject of chemical research, but it is to be regretted that the origin of the fluid investigated by the two former was unknown. ‖

Lassaigne separated the secretion into a heavy and a light oil.

Swarts found it to consist of a colourless portion boiling between 105° and 110° C., and a heavy yellow oil boiling between 195° and 200° C. In the residue he recognised a nitrogenous basic body which, from its volatility, he concluded to be either methylamine or ethylamine.

According to Loew, the secretion consists of at least two bodies, one of which is richer in sulphur than the other, possesses properties similar to the oil obtained from garlic (*Knoblauchöl*), gives with the metals salts, and is violently attacked by concentrated nitric acid; the other oil contains nitrogen and gives rise, under certain conditions, to a base which smells like trimethylamine.

The results of these investigators amount to very little from a chemical point of view. Nothing definite as to the real chemical nature of this secretion has hitherto been determined.

The material for this investigation was obtained principally from the State of Maine; a small quantity, however, came from Long Island. With one exception, it came in the sacs, which were emptied as soon as received. The quantity of fluid in the sacs varies

* From a letter to Prof. Abel.

† Cited by Swarts.

‡ Liebig's *Annalen*, 1862, cxxvii, p. 266.

Op. cit., p. 252.

‖ It is very probable that the secretion of the other members of the subfamily *Mephitinæ* differs very little from that of *M. mephitica*.

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greatly; some were found empty, others contained as much as 5 cubic centimetres, or even more if they were very much distended. I presume the age, vigour, and size of the animal, also the method employed in capturing, will explain this variation.

The length of time that elapsed before the sacs reached the laboratory varied from twelve hours to several days. In those cases where no especial care had been taken to prevent decomposition the sacs still gave no evidence of change. It is probable that the secretion preserves from immediate decomposition not only the tissue in contact with it, but also that in the immediate neighbourhood. No differences that could be attributed to decomposition were observed in any case, the odour and colour of the secretion from the different sacs being approximately the same.

Physical Properties of the Fluid.

Obtained from the sacs a few hours after the death of the animal, the secretion is a clear, limpid fluid, of a golden-yellow or light-amber colour, of a characteristic, penetrating, and most powerful odour, and having a specific gravity, at ordinary temperature, less than water (0·939).

The fluid filters readily, sometimes shows a decided greenish cast, and in the evening is said to be slightly luminous; but I myself have not been able to verify the last observation. After removal from the sac it turns gradually darker in colour, probably on account of a slight oxidation. It remains fluid at a temperature of —13° C.

There is generally present in the sacs to the extent of one gramme or more a yellowish amorphous body, having, as far as I have tested, an alkaline reaction, and giving the proteid tests. I take this substance to consist in large part of the *débris* of desquamated epithelial cells from the inner surface of the sac. Whether any chemical function is to be ascribed to this *débris* I have not yet been able to determine. Prismatic crystals have been observed when a little of the fluid has remained some time in contact with the amorphous body referred to. The secretion standing in a bottle often deposits a white flocculent substance.

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Chemical Properties of the Fluid.

The reaction of the fluid I found to be neutral; others have found it intensely acid;* but I can not reconcile this statement with the character of the chemical bodies found in the secretion. These substances without doubt belong to groups that have as one of their characteristic properties a neutral reaction.

The vapours of the fluid are highly inflammable and burn with a luminous flame, giving off sulphur dioxide.

Absolute alcohol, ether, and chloroform dissolve the fluid readily. Concentrated sulphuric acid dissolves it, but not without producing some changes, as the colour changes to a deeper hue. The body is thrown out of the alcoholic and concentrated sulphuric-acid solutions on dilution with water, in which it is practically insoluble. The odour in these solutions is but little changed.

A fifty-per-cent solution of sodium or potassium hydroxide dissolves the fluid partially, the peculiar odour disappearing almost entirely. From this solution the oil is liberated on dilution with much water; the addition of dilute sulphuric acid brings back the original odour. The same facts may be shown in the following way:

A strip of filter paper moistened with an aqueous solution of lead acetate and suspended over the natural secretion takes on a bright-yellow colour; held over the secretion that has been dissolved in a fifty-per-cent solution of potassium hydroxide, no change is noticed, though it may be in time somewhat darkened in colour; but if this potassium-hydroxide solution is acidified by the addition of sulphuric acid, the bright-yellow colour is again seen in the filter paper held over it. Paper moistened with copper sulphate is acted on in the same way, only that the colour is not so pronounced.

The dilute sulphuric-acid solution, or the aqueous solution, for it is soluble to a slight extent in water, reacts toward an iodine solution similarly to the corresponding solutions of alkyl-sulphides.† Bromine acts like iodine.

* Merriam. Mammals of the Adirondacks, p. 76.

† Abel. *Zeit. f. physiol. Chem.*, xx, pp. 271–273.

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An alcoholic solution of the secretion gives with alcoholic solutions of lead acetate, mercuric cyanide, mercuric, platinic, or auric chloride, for the most part amorphous precipitates. The lead compound is yellow in colour, the mercuric compounds are white, and the platinic and gold chloride compounds are orange. Mercuric oxide reacts either directly in the fluid, or in an ethereal solution of the same very energetically, as does also concentrated nitric acid; both form crystalline compounds. An alcoholic solution of ferric chloride gives with the corresponding solution of the secretion a light-green colour.

These physical and chemical properties just referred to warrant the assertion that one or more mercaptans, or thioalcohols, are present in the secretion.

Fractional Separation of the Secretion.

Being convinced of the presence of mercaptans in the secretion, fractional distillation suggested itself as the best method to use in their separation. Accordingly, the filtered secretion was distilled from a suitable bath. With the mercury completely in vapour, a clear, colourless fluid constituting about half of the secretion, distilled over between 100° and 130° C. The bath temperature was generally about 130°, never over 150° C. The temperature then fell, though the bath temperature was increased to 180° C. As there was no evidence of decomposition, it is natural to suppose that these bodies are in the original secretion, and not decomposition products. By this method we are enabled to separate the secretion into two sharply defined and nearly equal portions:

1. The more volatile portion, boiling between 100° and 130° C., and which for convenience we will designate A.

2. The less volatile portion, left after distilling off A, and which we will call B.

An apparatus so arranged as to detect any gas that might be given off under 100° C. gave a negative result. The three lower mercaptans—methyl (B. P.), 6°; ethyl (B. P.), 36°; normal propyl (B. P.), 67°; and iso-propyl (B. P.), 57° to 60° C.—were thus

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shown to be, in all probability, absent. We are therefore compelled to seek our mercaptans among those with boiling point near or over 100° and still under 130° C.

Fraction A.

A is a mobile colourless fluid lighter than water and having a very unpleasant penetrating odour, scarcely to be distinguished from that of the original secretion. It gives, without exception, all of the reactions of the original secretion.

After drying over calcium chloride for twenty-four hours it was distilled fractionally. Three fractions were obtained:

1. *a*, boiling between 100° and 110° C. and constituting about one half of A.

2. *β*, boiling between 110° and 120° and constituting one fourth of A.

3. *γ*, left in the bulb after distilling off the two former and constituting one fourth of A.

These three fractions—*a*, *β*, and *γ*—give the mercaptan reactions; that is, they unite with mercuric oxide, mercuric cyanide, and lead acetate to form compounds characteristic of the mercaptans. *β* was found to contain less sulphur than *a*, a result to be expected if a higher mercaptan were present. For the purpose of identifying the mercaptan in fraction *a*, several analyses were made.

Sulphur Analysis.

A quantitative determination, according to Carius, of the percentage of sulphur gave the following results:

I. 0·1535 gramme gave 0·3955 gramme $BaSO_4$.
II. 0·2453 " " 0·6250 " "

	Calculated for C_4H_9SH.	Found. I.	Found. II.
S	35·55 per cent.	35·37 per cent.	34·98 per cent.

In each analysis the sulphur is found too low. This may be readily explained by assuming the presence in small quantity of either amyl mercaptan, the sulphur contents of which are about thirty-three per cent, or of one of the higher sulphides.

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Lead Butyl Mercaptide, $(C_4H_9S)_2Pb$.

This compound was prepared by adding an excess of a saturated alcoholic solution of lead acetate to the alcoholic solution of the fraction *a*. It was obtained in beautiful glistening, yellow, rhombic plates, which, recrystallized from alcohol, washed with alcohol and ether, and dried *in vacuo* over sulphuric acid, gave the melting point 85° to 90° C. The compound is very unstable, decomposing readily into the corresponding mercaptan and sulphide of the metal. Possibly this instability may explain the great range of its melting point. Dilute hydrochloric acid as well as dilute sulphuric acid decomposes it, and more readily on heating. Prepared as given above, carbon, hydrogen, and lead determinations were made:

I. 0·2255 gramme gave 0·2106 gramme CO_2 and 0·0802 gramme H_2O.*
II. 0·2453 " " 0·2277 " " " 0·0858 " " *
I. 0·2033 " " 0·1584 " $PbSO_4$ = 53·22 per cent of Pb.
II. 0·1255 " " 0·0974 " " = 53·02 " "

	Calculated for $(C_4H_9S)_2Pb$.	Found. I.	II.
C	25·00	25·45*	25·31*
H	4·68	3·95*	3·89*
Pb	53·64	53·22	53·02

Mercuric Chloride Butyl Mercaptide, $(C_4H_9S.HgCl)$.

This body is precipitated as an amorphous compound, though inclined to crystallize, when alcoholic solutions of mercuric chloride and of fraction *a* are brought together. It is practically insoluble in cold alcohol, sparingly soluble in hot, from which it crystallizes in glistening white plates. Recrystallized from hot alcohol three times and dried *in vacuo* over sulphuric acid, it decomposes partially without melting when heated to 185° C. This compound, being stable in the air and having been recrystallized a number of times, gave results which compare more favourably with the theoretical figures.

I. 0·2746 gramme gave 0·1963 gramme of HgS = 61·62 per cent. of Hg.
II. 0·1658 " " 0·1192 " " = 61·94 " "

	Calculated for $C_4H_9S.HgCl$.	Found. I.	II.
Hg	61·63	61·62	61·94

* These results were obtained from the analyses of the lead mercaptide prepared as described on page 335

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Mercuric Butyl Mercaptide, $(C_4H_9S)_2Hg$.

When mercuric oxide is added to either *a* or its ethereal solution energetic reaction takes place, the oxide disappears, and a white, for the most part amorphous body, somewhat soluble in ether, is deposited. It can be crystallized from its ethereal solution and obtained in white rhombic plates. After having been dried *in vacuo* over sulphuric acid, it melted sharply at 94° to 95° C. to a clear colourless fluid. On cooling it solidified to a mass of crystals.

This compound, like most of the metallic derivatives of the mercaptans, decomposes readily; even during the process of recrystallization from ether, partial decomposition takes place; it turns gradually dark when kept for some time.

0·1108 gramme gave 0·1053 gramme CO_2 and 0·0386 gramme H_2O.

	Calculated for $(C_4H_9S)_2Hg$.	Found.
C	25·40	25·90
H	4·76	3·88

The difficulty of making a carbon determination directly with the fraction *a* will be understood when it is known that a portion of the carbon is deposited in the glass bulb before the entire fluid has volatilized. It was thus found impossible to burn all of the carbon.

The results of these analyses are sufficiently near the theoretical figures when considered together with the boiling point of *a* to convince, I think, the most sceptical that the greater part of this fraction contains one of the butyl mercaptans.

Primary normal butyl mercaptan is given as boiling at 97° C.* The boiling of *a* between 100° and 110° C. could be easily explained if we assume the presence in small quantity of some higher boiling body. This assumption would also explain in general the analytical results. I am inclined to believe in the presence of a higher mercaptan (say amyl mercaptan) rather than a sulphide. The other fraction of A will be taken up at some future time.

After the above analyses had been made I was fortunate enough

* When the mercury is completely in the vapour it will be found to boil a few degrees above 97°.

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to obtain a larger quantity of the secretion. This I treated in the manner already described as far as the separation of A and B is concerned. From this point A was not at first subjected to fractional distillation, but shaken up with a fifty-per-cent solution of potassium hydroxide in order to dissolve out the mercaptans, and then repeatedly shaken out with ether to remove any body not soluble in alkali. The results show, however, that when the first distillation is conducted carefully, A is practically free from any bodies outside of the mercaptans, there being very little oil or residue left after the ethereal solution is allowed to evaporate. Dilute sulphuric acid was then added to the alkaline solution, keeping it in an ice mixture, and the mercaptans, free of any possible sulphides, collected with ether. This ethereal solution was dried over fused calcium chloride for twenty-four hours and then, after filtration, subjected to fractional distillation; the same fractions were obtained as before; the boiling point and quantity of each fraction remained practically the same.

For comparison, the mercuric mercaptide obtained by adding mercuric oxide to an ethereal solution of α was made and recrystallized from ether. Dried in the usual manner, the melting point was found to be the same as the mercaptide previously described—94° to 95° C.

The lead mercaptide was also prepared in the usual manner and combustion analyses made. The results of these analyses have been already given under Lead Butyl Mercaptide, page 333.

Synthetical Formation of the Higher Mercaptans.

Several attempts were made to prepare synthetically a mercaptan having an odour and other properties similar to the fraction α or β.

The method employed was that used by Klason (*Berichte*, xx, p. 3904). The alcohols used were iso-butyl alcohol and iso-amyl alcohol. The results obtained with iso-butyl alcohol were not satisfactory; there appeared to be decomposition products formed when the alcohol was allowed to drop into the concentrated, and fuming sulphuric acid. The results obtained with iso-amyl alcohol, how-

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ever, were very satisfactory. About fifty grammes of pure iso-amyl mercaptan boiling between 115° and 120° C. were obtained which resembled in all outward appearances the fractions α and β already described, and its odour in concentration or dilution was hardly to be distinguished from that of these fractions or from that of the original secretion. It gave the mercaptan reactions, and could not by any of these reactions be distinguished from the fractions α and β of A.

I intend in the near future to prepare the normal mercaptans of these higher alcohols and compare them with the fractions of A.

Portion of the Secretion called B.

B has a neutral reaction, a dark-red colour, and an aromatic and less penetrating odour than either A or the secretion. The original colour of the secretion can be nearly brought back by adding a volume of alcohol or ether to B equal to A. This seems to indicate that very little decomposition had taken place in distilling off A.

B differs from A in containing nitrogen and in not reacting with mercuric oxide, mercuric cyanide, or lead acetate; it resembles A in giving all the other tests referred to under the secretion. I therefore infer the absence of mercaptans from B. This inference was proved to be correct, for upon shaking B with a fifty-per-cent solution of potassium hydroxide, and subsequently acidifying and shaking out with ether, a trace of an oil was obtained which failed to give the mercaptan tests.

The fact that B contains no mercaptans and still contains a large amount of sulphur, that it is insoluble in a fifty-per-cent solution of potassium hydroxide, that it reacts with mercuric chloride, also with iodine and bromine, and that it is violently attacked by concentrated nitric acid, justify me in predicting the presence of alkyl sulphides, or bodies closely related to them, in this portion of the secretion. I am inclined to believe, from certain tests that I have made, that these sulphur bodies are combined or mixed with some basic body containing nitrogen.

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B, together with a basic body isolated from it, will form the subject of another article.

The Odour of the Secretion.

It is hardly necessary to say that the odour of the secretion is exceedingly persistent, diffusible, and penetrating.

The essential principle in the secretion has been attributed to various substances, allyl sulphide and the lower mercaptans * being the ones usually suspected.

The great diffusibility and the penetrating property of the secretion is due to fraction A—that is, to the mercaptans; the permanency of the odour of the secretion is attributable to B, which tends to retain A and at the same time to modify its odour.

Amount of the Secretion that may be recognised by the Sense of Smell.

In order to test the sensitiveness of the olfactory nerves toward the fraction *a* and thus establish its identity with the more volatile and objectionable portion of the secretion, and also its relation to the mercaptans, the following experiments were performed:

A known weight of *a* was dissolved in 500 cubic centimetres of absolute alcohol. To 10 cubic centimetres of this solution another known quantity of alcohol was added, and from this last solution 2 or 3 cubic centimetres were taken, placed in a bottle similar to one used for washing gases, and blown out into a room by means of a bellows. Room capacity, 696 cubic metres.

The weighing took place in another building far from the room used, and the person who manipulated the bellows and mixed the portion of *a* with the air in the room had not been in the building where the secretion was kept and the weighings made. The mixing was continued for at least ten minutes by using large pieces of stiff paper as fans. At a given signal persons who had not previously been near the substance entered the room.

First Trial.—0·1 of a milligramme of *a* was mixed with the air of the room. The odour was very apparent to all on entering. My power to detect the mercaptans had, however, been so blunted by previous contact

* M. v. Nencki u. N. Sieber, *Sitzb. d. kais. Akad. in Wien*, Mathem. Classe, Mai, 1891, xcviii Abth. II. b., S. 1; Swarts, Leibig's *Annalen*, 1862, cxxiii, S. 266.

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with the substance that I was unable to detect in this dilution any odour whatever, although I could still detect mere traces of other odoriferous bodies. 0·1 of a milligramme diluted with 696 cubic metres of air allows $\frac{1}{6900000000}$ milligramme to each cubic centimetre.

Second Trial.—0·01 of a milligramme of *a* was distributed through the same room several days after the first trial and after the room had been thoroughly ventilated. The odour was faintly apparent to all on entering, but was not noticeable after they had remained even a short time in the room. After they had freed the nostrils of the odour in the open air they could again detect it on re-entering the room. In this trial the limit of dilution was approximately reached. In this dilution we have in every cubic centimetre $\frac{1}{69000000000}$ of a milligramme. Granting that 100 cubic centimetres of this air are necessary to make the odour evident in this dilution, we are able to recognise approximately $\frac{1}{6900000000}$ of a milligramme of *a* and about four times that quantity of the secretion.

E. Fisher * and Penzoldt have found by similar experiments that one is able to recognise $\frac{1}{4600000000}$ of a milligramme of ethyl mercaptan. These results prove the nose to be by far the most delicate testing apparatus known, for the smallest quantity of sodium that can be detected by the spectroscope is nearly two hundred and fifty times larger † than the amount required of ethyl mercaptan to rouse the sense of smell.

The similarity of ethyl mercaptan and the fraction *a* is very striking in respect to diffusibility and penetrating power, and it is no doubt this portion combined with the fractions β and γ which gives the secretion its all-pervading property.

Lack of material has prevented the further study of the physiological properties of the secretion. I take the liberty, however, of presenting certain facts that I have gathered from various sources in regard to these properties. in the hope that they will be of general interest.

The substance is a powerful anæsthetic,‡ and has also been used as an antispasmodic. When inhaled without the admixture of a large amount of air the victim loses consciousness, the temperature falls, the pulse slackens, and, if the inhalation were prolonged, the results would doubtless prove fatal.

When recently ejected, the fumes from this liquid are overpoweringly pungent and extremely irritating * to the air passages, and are said to be capable of producing œdema of the glottis. It is also said to be an efficacious remedy in certain spasmodic affections of the air

* Liebig's *Annalen*, 239, 131.

† Victor Meyer u. Jacobsen, Lehrbuch der organ. Chemie, p. 225.

‡ Skunk Perfume as an Anæsthetic. *Virginia Med. Month.*, August, 1881 vol. viii, No. 5, pp. 359, 360.

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passages. If any of this fluid finds its way into the eye it produces intense pain and sets up acute conjunctivitis, which commonly runs its course in a week or ten days. Moreover, we have reliable accounts of the entire loss of vision from this cause.

Swarts † complained while working with the secretion, especially when distilling with water vapour, of violent headaches and dysuria. His urine was voided with a burning sensation, and deposited a brown sediment which smelled like musk, as did also the urine. His perspiration for several days retained the odour of the secretion. Though I have worked with comparatively large quantities of the secretion and during a period of three months, I have failed to observe in myself the symptoms described by Swarts.

As far as the literature of this subject is known to me, the presence of the higher mercaptans in the animal organism has never before been demonstrated.

It is true that M. v. Nencki ‡ has found methyl mercaptan in the urine of those who had partaken freely of asparagus, and also among the gases given off during the putrefactive decomposition of the proteid; # and that methyl mercaptan has also been found in the urine as the product of a special bacterium,‖ and also among the gases of the large intestine; △ but all of these mercaptans, with the exception of the one arising from the use of asparagus, owe their origin to the putrefactive decomposition of proteids. The mercaptan in the skunk secretion, on the other hand, differs widely from the above, as far as its origin is concerned. It is without doubt a normal product of the cell activity of the gland.

The secretion of *M. mephitica* is not only remarkable for the quantity (about thirty per cent) of sulphur which it contains, and for this sulphur being in an unoxidized condition, but for the light it throws on the normal sulphur metabolism of the animal organism.

* Merriam, *op. cit.* † *Op. cit.*

‡ *Archiv f. exp. Pathol. u. Pharmakol.*, Bd. xxviii, pp. 206–209.

M. v. Nencki u. N. Sieber. *Monatsh. f. Chemie*, Bd. x, pp. 526–531.

‖ Karplus. Virchow's *Archiv*, Bd. cxxxi, pp. 210–222.

△ L. v. Nencki. *Sitzb. d. kais. Akad.* (Wien), Math.-Naturwiss. Classe, III. Abth., 98, pp. 437, 438.

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Abel * has isolated from the dog's urine a substance (ethyl sulphide) closely related to those found in this secretion, and has expressed the belief, founded on certain experiments, that it is a normal product of cell activity and not a product of putrefactive decomposition in the intestine. My results supplement his and give another instance of a highly unoxidized sulphur compound which is formed in the breaking down of the proteid molecule, and which is protected in some way from oxidation, or if oxidized, is subsequently reduced.

It is useless to speculate on the immediate forerunners of this substance or substances with the little knowledge we at present possess. Further examination of this interesting secretion, and further knowledge of sulphur metabolism, are required before we can theorize with much profit. It would, however, be of general interest, and no doubt increase our knowledge in this direction, to examine the urine of the skunk and determine the condition of its sulphur contents, also to conduct a number of feeding experiments for the purpose of determining the changes in the sulphur contents of the secretion and of the urine.

I will say in closing that after completing my investigation of the secretion I intend to carry on experiments along the lines just referred to.

* *Zeitschr. f. physiol. Chem.*, Bd. xx, S. 253.

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