following: overlapping plastic or heavy foil caps on tubes replace cotton or other porous plugs, both to minimize occluded oxygen and to maintain sterile tube lips. A pencil type electrocautery modified to heat a loop made of approximately 7 inches (18 cm) of 24 g nichrome wire to bright redness serves as an inoculating device. By means of the hose connector in the chamber, anaerobic jars carrying cold catalyst can be evacuated in the box and filled with 5% or more of CO_2 in forming gas. We have made convenient use of Mason jars for anaerobic incubation by trapping the chamber atmosphere in them with CO_2 added by appropriate evacuation and refilling of the whole system. A zoom dissecting microscope and focusing lamp mounted over the viewing port permit critical examination of plates in the anaerobic atmosphere.

With the aid of these methods we have been able to isolate spirochetes, apparently of several varieties, routinely by streaking directly from primary cultures(5) and transferring colonies to fresh medium. Such cultures fail to grow under equivalent conditions when streaked in air and incubated in anaerobic jars. Contaminated tube cultures can be purified by streaking in the box. More than 30 pure cultures of spirochetes have now been isolated either directly from human gingival scrapings or from exudates after passage of such scrapings through guinea pigs(8). Initial pure cultures in fluid medium have been stored at −78°C in an effort to prevent the changes, including increased O_2 tolerance, which seem to occur during *in vitro* passage.

Summary. Apparatus and procedures have been developed for bacteriological work based on a vacuum-tight glove box with lock, in which an anaerobic working atmosphere has been maintained by evacuation, followed by removal of residual and diffusing oxygen with hydrogen and a catalyst that does not require pre-heating. By these means anaerobic spirochetes have appeared regularly as surface colonies on agar streaked from primary cultures, and have been isolated routinely.

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Secretion of Nitrogenous Substances by the Pilocarpine-Stimulated Canine Prostate Gland.* (29707)

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Most of the available information on the composition of canine prostatic fluid was derived from studies on samples of fluid obtained by the systemic administration of pilocarpine(1-4). One characteristic of fluid thus obtained is that its composition varies during the course of secretion(2). Since such variation must of necessity reflect the nature of the secretory process, analysis of the composition of serial samples of the prostatic fluid obtained by pilocarpine stimulation should

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permit some insight into the mechanism of secretion. On this assumption the secretion of nitrogenous substances in prostatic fluid obtained by pilocarpine stimulation was studied by analyzing serial samples for dry weight, protein and nonprotein nitrogen and acid phosphatase activity.

Methods and materials. Fluid was collected from unanesthetized dogs with prostatic fistulas prepared according to procedures described by Mason *et al*(5). The dogs were castrated and were maintained during the period of these experiments on 5 mg of testosterone per day. Secretion of the prostate gland was stimulated by rapid intravenous injection of 700 μg/kg of pilocarpine hydrochloride while the dogs were restrained on a table in a standing position and the secreted fluid was collected in 4 fifteen minute serial samples. The doses of testosterone and pilocarpine used were found to be particularly suitable for the study of prostatic secretion in castrated dogs(6,7). An interval of at least 3 days was allowed between collections from a given animal.

Thirty-two samples of prostatic fluid collected from 4 dogs were centrifuged and analyzed for dry weight, total and nonprotein nitrogen and acid phosphatase activity according to procedures described by Rosenkrantz *et al*(3); protein nitrogen was then calculated as the difference between total nitrogen and nonprotein nitrogen. When limited by the volume of the sample, analyses were conducted in the following order of priority: acid phosphatase, total nitrogen, dry weight, and nonprotein nitrogen (NPN). In all, the volumes collected permitted 23 complete and 9 partial analyses.

Results. 1. Stimulation of prostatic secretion by pilocarpine. The rate of prostatic secretion without external stimulation in these unanesthetized dogs was practically nil (a few drops an hour at the most). Pilocarpine markedly increased the rate of prostatic secretion and concomitantly produced varying degrees of salivation, lacrimation, emesis, defecation and urination. The volumes of the samples of prostatic fluid as well as the results of the chemical analyses are shown in Table I. In addition, the Table also

TABLE I. Secretion of Nitrogenous Substances by the Pilocarpine-Stimulated Canine Prostate Gland.
Each value represents the mean ± S.E. (number of dogs in parentheses).

Collection period (min after pilocarpine)	Volume, ml	Dry wt, mg/ml	Nonprotein nitrogen, mg/ml	Acid phosphatase activity, mg PO_4/hr/ml	Protein nitrogen, mg/ml	Protein,* mg/ml	Protein-free dry wt,† mg/ml
I (0-15 min)	19.8 ± 4.1 (4)	33.3 ± 3.6 (4)	.254 ± .030 (4)	13.17 ± 1.39 (4)	3.574 ± .592 (4)	22.4 ± 3.7 (4)	10.9 ± .3 (4)
II (15-30 ")	8.2 ± 2.7 (4)	19.9 ± 2.0 (4)	.244 ± .019 (4)	7.74 ± 1.90 (4)	1.349 ± .313 (4)	8.4 ± 2.0 (4)	11.5 ± .9 (4)
III (30-45 ")	4.8 ± 1.4 (4)	20.3 ± 1.9 (4)	.237 ± .015 (3)	7.48 ± 1.62 (4)	1.580 ± .452 (3)	9.9 ± 2.8 (3)	9.9 ± .2 (3)
IV (45-60 ")	4.0 ± 1.2 (4)	21.4 ± 2.1 (4)	.251 ± .013 (4)	7.90 ± 1.51 (4)	1.498 ± .557 (3)	9.4 ± 3.5 (3)	11.5 ± 1.3 (3)
Significance of the difference between means determined by the Newman-Keuls sequential range test(8).							
Mean square residual (d.f.)	8.472 (9)	13.454 (9)	0.0013 (7)‡	1.255 (9)	0.4833 (7)‡	18.874 (7)‡	2.828 (7)‡
I *vs* II	P<.01	P<.01	N.S.	P<.01	P<.05	P<.05	N.S.
I *vs* III	P<.01	P<.01	"	P<.01	P<.05	P<.05	"
I *vs* IV	P<.01	P<.01	"	P<.01	P<.05	P<.05	"
II *vs* III	N.S.	N.S.	"	N.S.	N.S.	N.S.	"
II *vs* IV	"	"	"	"	"	"	"
III *vs* IV	"	"	"	"	"	"	"

* Calculated assuming nitrogen represents 16% of the dry wt of the protein (see text for data supporting this assumption). † Dry wt less protein wt.
‡ For collection periods III and IV the mean value for three dogs was substituted for a missing value.

shows the corresponding calculated values for weight of total protein assuming protein nitrogen to be 16% of total protein weight. Protein-free weight was then calculated as dry weight minus total protein weight. The Newman-Keuls sequential range test(8) indicated that volume, dry weight, acid phosphatase activity, protein nitrogen and total protein content of the first sample was significantly greater than each of the following samples, and that there were no significant differences among the last 3 samples. There were no differences among the 4 samples for NPN content and calculated protein-free dry weight. The higher total dry weight of the first sample was obviously due solely to its higher protein content.

2. Secretion of protein. A plot of the protein nitrogen content of each sample, irrespective of animal or time of collection, against its volume revealed no relationship between these 2 variables and the linear correlation between them was not significant ($r = 0.398$; $P > 0.05$; $n = 23$). The secretion of protein apparently was relatively independent of the secretion of water.

In contrast, protein nitrogen content and dry weight of the samples were closely related. The correlation coefficient was highly significant ($r = 0.939$; $P < 0.01$; $n = 23$). The 2 regression equations, fitted according to the method of least squares(9) by ascribing all the experimental errors first to determination of protein nitrogen and then to determination of dry weight, were (protein nitrogen) $= -1.70 + 0.158$ (dry weight) and (dry weight) $= 11.66 + 5.95$ (protein nitrogen) (dimension as shown in Table I). These equations indicate that the samples of prostatic fluid could be considered as mixtures of a protein-free solution with a mean dry weight between 10.8 and 11.7 mg/ml (intercept of each line) and variable amounts of protein with a mean nitrogen content between 15.8 and 16.8% (slope of each line). It was on this basis that a protein nitrogen content of 16% was assumed in calculating protein contents and protein-free weights shown in Table I.

3. Secretion of acid phosphatase. There was no apparent relationship between the 2 variables when acid phosphatase activity was plotted against volume and the coefficient of linear regression was not significant ($r = 0.168$; $P > 0.05$; $n = 31$). However, acid phosphatase activity was related to dry weight. The correlation between these variables was highly significant ($r = 0.827$; $P < 0.01$; $n = 31$) and the regression equations were (acid phosphatase activity) $= -1.49 + 0.44$ (dry weight) and (dry weight) $= 9.82 + 1.55$ (acid phosphatase activity).

The acid phosphatase activity of the various samples was also related to protein nitrogen content. The calculated correlation coefficient was highly significant ($r = 0.864$; $P < 0.01$; $n = 23$). The two regression lines were (acid phosphatase activity) $= 3.77 + 2.66$ (protein nitrogen) and (protein nitrogen) $= -0.50 + 0.28$ (acid phosphatase activity). The intercept of the second equation was (protein nitrogen) $= -0.50 \pm 0.38$ (S.D.) and was not significantly different from the origin ($t = 1.316$; $P > 0.05$) while, in contrast, the intercept of the first equation was (acid phosphatase activity) $= 3.77 \pm 0.86$ (S.D.) and this was significantly different from the origin ($t = 4.368$; $P < 0.001$). The mean acid phosphatase activity of these samples was between 2.7 and 3.6 mg PO_4/hr per mg protein nitrogen (slopes of the 2 lines, b_{yx} and $1/b_{xy}$).

4. Secretion of nonprotein nitrogen. No apparent relation was seen upon plotting the NPN content of each sample against its volume; and, in addition, the linear correlations between these variables was not significant ($r = 0.032$; $P > 0.05$; $n = 23$). On the other hand, there were statistically significant but not striking correlations between NPN content and protein nitrogen content ($r = 0.457$; $P < 0.05$; $n = 23$) and the dry weight ($r = 0.474$; $P < 0.05$; $n = 23$).

Discussion. The results presented show that the rate of secretion of prostatic fluid was maximal soon after administration of pilocarpine and declined subsequently. The first 15 minute sample contained considerably more protein that the 3 later samples as indicated by a much higher protein nitrogen content and acid phosphatase activity. Because of its higher protein content the first sample

also had a much higher dry weight than the subsequent 3 samples.

There are at least two possible explanations for the higher protein content of the first 15 minute sample. One explanation is that a portion of the initial sample of fluid represents the fluid which had been formed by the gland and had remained within the collecting ducts of the gland until released by pilocarpine administration. If this fluid were to contain large amounts of protein, due perhaps to reabsorption of water and nonprotein substances through the walls of the tubular ducts of the gland, then the first sample collected would contain more protein than the subsequent samples. A second possible explanation is that the stored or preformed protein was released from the secretory cells upon stimulation with pilocarpine and appeared in the first sample while subsequent samples contained only the newly-formed proteins. If this were true the protein content of the first sample might reflect the capacity of the prostate to store proteins while the protein content of the later sample might reflect the ability of the prostate to synthesize protein. The results of the present experiments do not indicate which, if either of these explanations, may be correct.

Whatever the cause, the variations in composition of prostatic fluid which have been observed in the present experiments provide some information about the nature of prostatic secretion, particularly the secretion of proteins. A number of proteins have been found in canine prostatic fluid, including albumins, globulins, nucleoproteins, while the enzymes which have been detected include acid and alkaline phosphatase, amylase, β-glucuronidase and at least 3 proteolytic enzymes(1-4). The results of the present study reveal that the mean nitrogen content of these proteins is between 15.8 and 16.8% of their dry weight and that their secretion is not directly related to the secretion of water.

These experiments have revealed a highly significant correlation between the acid phosphatase activity and the protein nitrogen content of prostatic fluid. The data do not allow the conclusion that acid phosphatase represents a constant fraction of the secreted protein but do not exclude this possibility. The data do clearly indicate that the secretion of acid phosphatase reflects protein secretion in general.

Summary. Prostatic fluid secreted by castrated, testosterone-treated dogs in response to intravenously administered pilocarpine was collected in four 15 minute samples and analyzed for dry weight, protein and nonprotein content and acid phosphatase activity. The volume, dry weight, protein content and acid phosphatase activity of the first sample were greater than the subsequent 3 samples which in turn did not differ from each other. The higher dry weight of the first sample was attributable solely to its higher protein content. Significant correlations were found between the protein nitrogen content and the dry weight, the acid phosphatase activity and the NPN content and between the dry weight and the acid phosphatase activity and NPN content of the collected samples.

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