These results and earlier observations lead to the speculation that phenomena of this sort are involved in initiation of motility of spermatozoa at ejaculation. Spermatozoa are inhibited by high p CO_2 and high K concentration in the epididymis. The enzyme or enzymes of spermatozoa responsible for cleavage of sulfide from the substrate, probably also from the cells, are probably activated by the citrate added to cells on admixture of accessory gland fluids at ejaculation. After activation, the sulfide diffuses from the cells to be converted to sulfite. The CO_2-release stimulated by these compounds, along with dilution of K by sodium chloride and uptake of hexose, result in reversal of the metabolic inhibition found in the epididymis.

Summary. Neither cysteine, glutathione nor sulfate exhibit a measurable effect on anaerobic glycolysis nor on its inhibition by high p CO_2. Sulfide, at higher than physiological levels, and sulfite, in amounts found in semen, stimulate anaerobic glycolysis and reverse inhibition of glycolysis by CO_2.

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Effect of Plasma from Wounded Donors on Nitrogen and Sulfur Excretion.* (24878)

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Several laboratories have presented data which seem to indicate that injured cells produce or cause production of a factor or factors having ability to alter rate of healing of wounds. These data have been obtained by observing the effects of extracts of crushed tissues when applied directly to healing wounds or when administered parenterally to wounded animals(1-3). Sandblom(4) and Auerbach(5) reported that the presence of injured tissue in the body causes production of factors appearing in the circulation, which affect rate of healing. They also observed that, when 2 wounds are consecutively inflicted in the same animal, rate of healing of the second wound appears affected by presence of the previously inflicted wound. Metabolism of nitrogen and sulfur have been shown to be markedly altered during healing of experimental wounds. As a result of wounding, the amount of urinary nitrogen increases sharply, while at the same time there is a relative retention of sulfur compounds by the wounded animal(6-9). At this time also, proteins rich in cystine and methionine are synthesized in regenerating wound tissue; these newly synthesized proteins must be considered to be distinct from collagen, since this latter protein contains no cystine and very little, if any, methionine(14). In this study, the effects of factor(s) released into blood by the stimulus of wounding, as they affect me-

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tabolism of nitrogen and sulfur, were investigated.

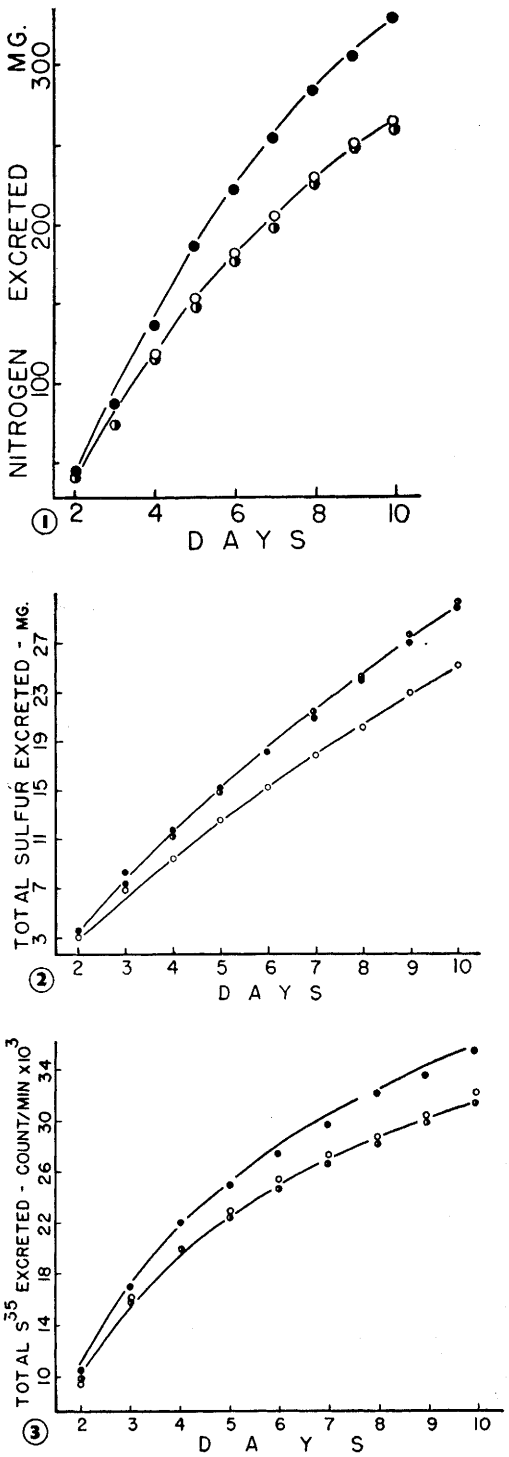
Methods. Adult female white rats weighing 220 ± 20 g were used. Wounded animals used as source of plasma, and animals for the metabolic studies were kept in individual metabolism cages during experiment. Rats used as blood donors were fed commercial stock diet, *ad lib.*, while those used in metabolic studies were given 8 g of protein-free diet daily(12). Each experimental rat completely consumed this diet in 24 hours. Plasma from donor rats was obtained as follows. After anesthetizing rats with nembutal, half of the animals were wounded by excising a circle of skin 4 cm in diameter, through the fascia to the muscle, in region of neck and shoulder blades. On day following wounding, and for 4 to 6 days thereafter, 2-4 ml samples of blood were obtained from each animal by heart puncture. Heparin was used to inhibit clotting. Plasma was immediately centrifuged free from cells and dried by lyophilization. The dried plasma was pooled and stored until used, in a desiccator at 5°C. Comparison of the effect of plasma from normal and wounded animals, on nitrogen and sulfur metabolism was undertaken by using 3 groups of 8 rats. These animals were acclimated to the protein-free diet for 3 days prior to start of experiment. Thereafter, each rat in Group I was given intraperitoneally 50 mg of dried plasma obtained from wounded rats (redissolved in 0.5 ml of water); animals in Group II were given the same amount of plasma from normal unwounded donors, while those in Group III were injected with 0.5 ml of 0.15 *M* NaCl solution. These solutions were administered twice daily at about 12 hour intervals. Each rat was also given an isotonic saline solution containing 5 x 10^6 counts/minute of S^{35} labeled L-cystine. Labeled amino acid was given only once at time of first administration of plasma. Administration of pooled plasma and saline were continued for 10 days. During experimental period, 24-hour urine samples were collected and analyzed for nitrogen, total sulfur and S^{35}. Nitrogen was determined by micro-kjeldahl method; sulfur by modification of the method of Toennies and Bakay(10), the digestion of samples being carried out with concentrated nitric acid and 30% hydrogen peroxide. S^{35} activity was determined by counting $BaSO_4$ samples at infinite thickness in Tracerlab SC-16 windowless gas-flow counter.

Results. Comparison of total urinary nitrogen excreted by rats in 3 experimental groups is plotted in Fig. 1. Animals receiving plasma from wounded donors and those given isotonic saline excreted the same amount of nitrogen. This level of excretion was significantly less than nitrogen output of rats given plasma from normal donors. Since plasma nitrogen administered to animals in Groups I and II was essentially the same, the difference in nitrogen excretion must be attributed to some cause other than nitrogen intake. Differences in nitrogen output are inexplicable unless one considers the possibility of qualitative differences in plasma obtained from wounded and normal donor animals. It then seems probable that these effects may be brought about by some substance of a hormonal nature.

The data on excretion of sulfur (Fig. 2) indicate that significantly less was excreted by animals receiving plasma from wounded rats as compared to those receiving normal plasma. This situation is somewhat analogous to that observed when excretion of sulfur by wounded rats is compared to that in unwounded control rats(13).

In Fig. 3 is presented excretion of S^{35} by the 3 groups of rats. Again, significantly more of the isotope is excreted by rats given normal plasma than by the other 2 groups. Administration of plasma from normal rats seems to have stimulated an increased excretion of S^{35}-labeled metabolites, as well as nitrogen, while plasma from wounded donors appears to cause retention of labeled sulfur. It is possible that differences in the metabolic picture displayed by rats in Groups II and III may be due to additional protein available to animals in Group II.

On 10th day of experiment, the rats were killed and weight of various organs among the 3 groups was compared. No significant difference in organs from each group was



Open circles, Group I, received plasma from wounded donors; *solid circles,* Group II, received normal plasma; *half-solid circles,* Group III, received isotonic saline.

TABLE I. Organ Weights of Rats Given Plasma from Normal and Wounded Rats.

Group	Kidney† Organ wt, g	Kidney† g/100 g body wt	Liver† Organ wt, g	Liver† g/100 g body wt
I *	1.577	.828	6.01	3.20
II	1.860	1.000	8.64	4.58
III	1.451	.797	6.50	3.57

* Group I, 100 mg of plasma solids/rat/day from wounded donors. Group II, 100 mg of plasma solids/rat/day from normal donors. Group III, 1.0 ml of saline/rat/day.

† Statistical difference between Group I and II and between II and III: $p < 0.01$. No significant difference between Groups I and III.

found except in the case of liver and kidneys, which were heavier in animals of Group II (normal plasma) than in the other groups (Table I). It seems probable that the greater weight of these organs, as well as the greater excretion of nitrogen and sulfur by rats receiving normal plasma reflects some degree of loss of muscle protein. The possibility that stress of repeated withdrawals of blood may be sufficient to cause increased plasma ACTH level(11) cannot be discounted. Thus, it may be considered that administration of normal plasma resulted in changes in metabolism of protein deficient animals, and that these changes were counteracted by some substance in plasma obtained from wounded animals.

Summary. Rats, on a protein deficient diet, were given reconstituted plasma from wounded donors. These animals excreted less nitrogen and sulfur than did comparable rats given plasma from normal donors. Similar results were obtained on excretion of S^{35}, administered as labeled cystine. After 10 days, liver and kidney of rats receiving normal plasma were significantly heavier than in rats getting plasma from wounded animals. The data appear to indicate that plasma from wounded rats contains a factor(s) which affects metabolism of nitrogen and sulfur.

FIG. 1. Total cumulative output of nitrogen in mg/day.

FIG. 2. Total cumulative excretion of sulfur in mg/day.

FIG. 3. Total cumulative excretion of S^{35} in counts/min. $\times 10^3$, corrected for decay to time of administration. S^{35} was administered in form of cystine-S^{35}.

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Bovine Ocular Squamous Cell Carcinoma II. Tissue Culture Studies of Papilloma.* (24879)

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Studies on pathological anatomy of bovine ocular squamous cell carcinoma demonstrated eosinophilic, cytoplasmic inclusion-like bodies in cells of both benign precursor and malignant lesions(1). Plaque and papilloma, benign precursor lesions, exhibited bi-laterality, disappearance and reappearance, which suggested a virus may be involved in the etiology of this disease. Tissue culture studies of cells derived from plaque lesions(2) have shown characteristic changes suggestive of a filterable agent. As a result of these preliminary findings, tissue culture studies of papilloma were undertaken to determine whether similar changes also occur in cells from papilloma grown *in vitro*.

Materials and methods. The method of removal of the lesions, treatment for transportation to the laboratory, subsequent treatment in laboratory and methods used for detailed study of cellular outgrowths from lesions have already been described(2,3,4).

Results. Phase contrast microscopy of cells derived from papillomata growing in specially designed chambers(2) has confirmed and extended preliminary observations on stained cultures in T-30 culture flasks examined at low magnifications by bright field microscopy. The cells derived from papillomata are epithelioid in character, relatively uniform in size measuring 0.01 mm in diameter, and frequently multinucleated (Fig. 1). Occasional large cells are seen as much as 0.03 mm in diameter; these cells are always multinucleated. The cells exhibit well marked tonofibrils, mitochondria long and interlacing are well developed (Fig. 2). Pinocytosis is marked, many cells showing cytoplasmic fimbriae (Fig. 3). Cells frequently exhibit vacuolisation of cytoplasm. Such cells occur in scattered foci. Vacuoles are found in the perinuclear zone and contain dense homogeneous material (Fig. 4). Cytoplasmic inclusions, irregular in outline, and not associated with presence of vacuoles also occur (Fig. 5). The nuclei contain multiple nucleoli of variable size and shape, and occasionally show margination of chromatin (Fig. 6). As in cells derived from plaque lesions, vacuolisa-

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