of the Rous chicken sarcoma. After standing for 30 minutes, the mixture was centrifuged, and the supernatant fluid decanted, neutralized and concentrated *in vacuo* to ½ its original volume. To 2 cc. of the concentrated supernatant fluid was added 0.5 cc. of filtrate, and the mixture allowed to stand for 30 minutes at room temperature. Such a mixture when injected into chickens fails to produce tumors in 75% of the inoculations. If, however, the mixture is brought to pH 4, and the precipitate extracted at pH 8, the active agent is recovered, for the extract is now able to induce tumor growth. Similar results were obtained when blood was used instead of the supernatant fluid.

This experiment has been repeated several times, and the agent has been recovered in almost every instance. It is evident then that the tumor producing agent in these mixtures is not destroyed *in vitro* by the inhibitory substance. That we should fail to recover it in every instance is not strange, since the amount of the agent used in each mixture is not large, and we have never been able to extract all of it from the precipitate.

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Effect of Sulfhydryl Compounds on Regeneration in Podarke Obscura.

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From his studies on the toxicity of Pb ions in cell division in onion root tips, which he explained on the basis of the binding and eliminating of a SH-group, and from his experiments designed to show the effect of SH-groups on rate of growth depending on cell multiplication, as in growing onion root tips, Paramecia cultures, and healing of wounds, Hammett[1] postulates his theory that the SH is the "mitotic hormone" and the wound hormone or essential chemical factor in cell proliferation following trauma.

With the thought that a specific mitotic hormone would at least accelerate, if not also actually increase, regeneration since the latter depends on cell division and proliferation in its earlier stages, we performed a series of experiments on regeneration of the polychaete

[1] Hammett, F. S., *Protoplasma*, 1929, **7**, 297.

worm, *Podarke obscura.* This animal was chosen because one of us (S. M.) has had extensive experience with this material and had previously made a long study of its regenerative process.[2]

The procedure was simple. Under a magnifying glass we cut away the posterior portion of the worm (which, by the way, never regenerates a head) aiming always to effect the severance at practically the same relative level. The cut must be made clean so that the wound closes up smooth and without any adhering shreds of necrotized tissue. All these details of operation are extremely important, if one is to insure a normal progress of regeneration. The same number of operated worms were distributed in a series of fingerbowls containing 90 cc. sea water with or without the tested reagents. In some of the experiments we followed a preferable course of leaving the worms in pure sea water until the wound had closed over and the first signs of initiation of the regenerative process appear. For a statistical study it is better if the reagents whose effect is being studied are applied to animals all of which have an even start. This eliminates from counts specimens which had begun their regeneration with some handicap and continue to lag behind. The control animals of each series were kept in pure sea water, while the others were in sea water containing various concentrations of the studied sulfhydryl compound. The water was changed daily and the solutions were made up fresh every day. The fingerbowls pertaining to one series of experiments were stacked together and kept in diffuse light, thus insuring uniformity of temperature and light conditions.

The rate of regeneration was determined by the number of new segments which were formed. The worms were examined daily with a binocular microscope. One familiar with the manner in which these animals regenerate can distinguish the new segments at a very early stage of their formation. The study, to be limited to the process of cell proliferation, can not be much prolonged, because after the first few segments have been formed the growth by increase in size becomes the predominant feature.

The sulfhydryl concentration of the various test solutions was determined daily. A solution of the particular sulfhydryl compound was prepared and its SH concentration was measured by treating this with an excess of a standard iodine solution and titrating back the unused amount of iodine with 0.01 N Sodium thiosulfate. The desired concentration of SH was then produced by proper dilution with sea water. The further dilutions in the finger-

[2] Morgulis, S., *J. Exp. Zool.*, 1909, 7, 595.

bowls were effected simply by arranging the series in such a way that the successive concentrations formed a geometrical progression.

We studied the effect on the rate of regeneration of the following compounds: thio-p-cresol, thio-phenol, thio-glycollic acid, and cystine. The former 2 compounds were also controlled by means of p-cresol and phenol. Cystine was used because from Hammett's experiments, although he seems to attribute to SH the hormonal

TABLE I.
Percent of total number of animals.

Number days	Stage regeneration	Control sea water	Thio-p-Cresol Mgm. S per cc. of sea water				p-Cresol Gm. per cc. sea water				
			2.6×10^{-6}	2.6×10^{-7}	2.6×10^{-8}	2.6×10^{-9}	1×10^{-7}	1×10^{-8}	1×10^{-9}	1×10^{-10}
3rd	1 Segment	92	0	50	75	90	0	50	90	100
4th	2 "	75	Dead	29	64	54	0	30	45	90
	3 "	25		0	0	18		10	0	10
6th	4 "	17		38	64	90	0	33	10	10
	5 "	57		0	9	0		43	78	10
8th	5 "	33		60	20	45	0	37	30	10
	6 "	33		0	30	23		13	30	50

TABLE II.
Percent of total number of animals.

Number days	Stage regeneration	Control sea water	Thio-glycollic Acid. Mgm. S per cc. of sea water							
			2.5×10^{-4}	2.5×10^{-5}	2.5×10^{-6}	2.5×10^{-7}	2.5×10^{-8}	2.5×10^{-9}	2.5×10^{-10}
2nd	1 Segment	100		100	100	100	100	100	100
3rd	2 "	100		100	100	88	94	90	100
4th	3 "	100	100	100	100	100	100	100	100
5th	5 "	58	0	28	55	58	55	60	56
6th	6 "	83	0	29	59	70	72	80	62
7th	7 "	67	Dead	33	41	56	73	61	62

property, it is obvious that he finds both the reduced and oxidized sulfur group effective.

The results are recorded in the accompanying tables, giving for each day the percentage of animals which have reached a certain maximum number of segments in the control and in the various concentrations of the tested solutions. Since those animals lagging behind are thus not included, the percents do not always add up to 100.

The reported data hardly require comment. As can be seen, there is no evidence of a cumulative advance in the regeneration of experimental animals as compared to the controls. In many cases the concentrations of the tested SH compounds were such as to be even toxic. Even in non-toxic concentrations, however, we found either that worms in the test solutions actually regenerated slower than the controls or at best about the same rate. If a slight advantage seems to appear one day, it is alternated with a disadvantage the next.

The sharp discrepancy between our results and those reported by Hammett calls perhaps for some critical evaluation. Owing to limitation of space it will be merely pointed out that Hammett's method of calculation applied to his experiments with the SH compounds is theoretically erroneous. The difference between the control and test root tips is not expressed on the basis of the absolute findings but as a difference of the percent of increment in both the control and treated roots. This tends to grossly exaggerate the actual differences. Considering that the changes reported are not sufficiently striking to justify the claim that sulfhydryl compounds in general constitute the mitotic hormone, they really become quite insignificant when recalculated as the percent of increment rather than as the *difference in percents of increment,* as Hammett does.

The counting of mitotic divisions in growing root tips is also subject to the serious criticism that since mitosis is not a continuous process but occurs in cycles one must be sure, of course, that the comparison is made at exactly the same phase of the cycle in both control and test objects.

The experiments with Paramecia are likewise very inconclusive. No check has evidently been kept on the pH of the culture, which is a serious omission. The differences in the *number of divisions* which the untreated and treated Paramecia undergo are not sufficient to draw any conclusions, considering the extreme variability of the material even under ordinary conditions. Finally, the experiments with thio-cresol[3] which seem to show its stimulating action

[3] Reimann, S. P., *J. Am. Med. Assn.*, 1930, **94**, 1369.

upon cell proliferation as manifested in the healing of extensive wound surfaces treated with this substance fail to take into account that the use of the dilute alcohol as solvent may be actually responsible for the good clinical results. Surgeons are well familiar with the beneficent effect of dilute alcohol wet dressings.

Since this paper was written two articles came to my attention which should be mentioned here. Gaunt[4] studying the division of fresh water snail eggs under the influence of cysteine failed to find any stimulating action. Secondly, Voegtlin and Chalkley[5] in a splendid investigation on the effect of reduced and oxidized glutathione on the division of *Amoeba proteus* have brought forth evidence of a stimulating action on *nuclear* division, which, however, is conditioned by the state of "maturation" of the cell, *i. e.,* upon some purely intrinsic factors, the nature of which is entirely unknown. These results are extremely significant, but they have little bearing upon Hammett's simplified scheme of mitosis of which SH is the key.

Summary. Neither thio-p-cresol, thio-phenol, thio-glycollic acid or cystine accelerates the process of regeneration in the *Podarke obscura.*

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Effect of Oestrin and Lutein Combinations on Uterus of the Mouse.

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It is well established that the physiological effects of the oestrin or follicular hormone of the ovary, when injected into various animals, will cause enlargement of the uterus. Allen and Doisy have very recently reviewed literature on the subject.[1] Advantage has been taken of this property of the ovarian hormone in a most interesting way by A. C. Siddall for the diagnosis of pregnancy and also for the evaluation of commercial ovarian products.[2, 3] From a study of a large number of mice, Siddall found that the ratio of the weight of

[4] Gaunt, R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 660.

[5] Voegtlin, C., and Chalkley, H. W., *Public Health Reports,* 1930, **45**, 3041.

[1] Allen and Doisy, *Physiol. Rev.*, 1927, **8**, 60.

[2] Siddall, *J. Am. Med. Assn.*, 1928, **90**, 380.

[3] Siddall, *J. Am. Med. Assn.*, 1928, **91**, 779.