caused by kaolin, but fails to inhibit the oedema produced by serotonin, histamine or PVP[9]. It appears thus that the specificity of the natural anti-inflammatory activity is similar to that of phenylbutazone in these type of oedemas.

ROBINSON et al.[7,8,13] have described an anti-inflammatory factor from exudates of animals subjected to irritation by implanting polyester sponges. Our material showed some similarity to theirs, since on gel filtration both substances behave as macromolecules. ROBINSON et al.[7,8,13] investigated the effect of their natural anti-inflammatory factor only on carrageenin oedema, so it is still debatable whether these 2 factors are similar or identical in their pharmacological properties. The hypothesis that the natural anti-inflammatory factor is synthesized in the liver[14] and transported to the diseased site during inflammation[8], requires further investigation. However, our material possesses a higher molecular weight than the anti-inflammatory factor isolated by HUBER et al.[15] from bovine liver. Further pharmacological characterization of the anti-inflammatory material obtained from pouch is in progress.

Table II. Anti-inflammatory activity of retentate and dialysate of water-soluble components of pouch material

Components tested	Dose[c] (mg/kg)	Inhibition of oedema[a] (%)
Retentate	50	29 (11–44)[b]
	100	50 (34–65)
	200	49 (33–64)
	400	64 (48–78)
	800	87 (73–101)
Dialysate	500	32 (10–50)

[a] Oedema was induced by kaolin. [b] In brackets: 95% fiducial limits. [c] Test material was dissolved in physiological saline and a volume of 1 ml/100 g body weight of rat was administered i.p.

Table III. Effect of the natural anti-inflammatory factor of pouch material on different types of oedema

Oedema inducer	% inhibition caused by retentate[a] (100 mg/kg)
Carrageenin	21 (11–31)
Kaolin	63 (49–77)
Histamine	0
Serotonin	0
Polyvinylpyrrolidon	0

[a] Obtained by dialysis of water-soluble pouch material.

Zusammenfassung. Eine hochmolekulare Verbindung wurde aus Exudaten von Granulombeuteln von Ratten gereinigt. Diese Substanz, i.p. an Ratten verabreicht, hemmt das durch Kaolin oder durch Carrageenin erzeugte Pfotenoedem, nicht dagegen das durch Histamin, Serotonin oder Polyvinylpyrrolidon erzeugte Oedem.

I. L. BONTA[17], N. BHARGAVA and C. J. DE VOS

Department of Pharmacology, N.V. Organon, Oss (The Netherlands), 30 December 1969.

[13] M. E. J. BILLINGHAM, B. V. ROBINSON and J. M. ROBSON, Br. med. J. *2*, 93 (1969).
[14] E. J. SARCIONE and A. E. BOGDEN, Science *153*, 547 (1966).
[15] W. HUBER, T. L. SCHULTE, S. CARSON, R. E. GOLDHAMMER and E. E. VOGIN, Abstr. of paper 7th Ann. Meet. Soc. Toxicol., Washington D.C., March 4–6, 1968; Toxicol. appl. Pharmacol. *12*, 308 (1968).
[16] Acknowledgements. We thank Miss RITA FROM, Mr. T. TIJS and Mr. H. KROLS for excellent technical assistance. Mr. D. W. R. HALL corrected the text linguistically.
[17] Present address: Department of Pharmacology, Medical Faculty, Rotterdam (Nederland).

A Role for Glutathione in Muscle Contraction

The development of rather specific agents for the intracellular oxidation of glutathione, GSH, to GSSG has permitted the investigation of the function of GSH in many biological systems[1–6]. We now report a heretofore unsuspected linkage of GSH to muscle contraction.

The thiol-oxidizing agent used in the present experiments is diamide, $(CH_3)_2NCON{=}NCON(CH_3)_2$, which reacts with GSH according to the equation[2]:

$$2GSH + (CH_3)_2NCON{=}NCON(CH_3)_2 \rightarrow GSSG + (CH_3)_2NCONHNHCON(CH_3)_2$$

The half-life for the reaction of diamide with intracellular GSH under the conditions used would be 1 sec or less (W. CORREA, unpublished observations).

Primary cultures of muscle cells (*Rattus rattus norvegicus*) in medium 199 were supplied by Dr. DAVID YAFFE (Department of Cell Biology)[7]. 2-week-old cultures exhibited strongly contracting fibres. Experiments were begun by adding a small volume of diamide solution in isotonic saline to the culture medium (25 λ: 3 ml) followed by rapid swirling. Observations were made under a dissecting microscope. After 80–120 sec, the medium was aspirated from the plate and replaced with fresh 199 medium. The plates were examined immediately after medium change and then periodically after storage in an incubator at 37 °C.

Treatment of actively contracting fibres with a $5\times 10^{-4}M$ diamide solution led to complete cessation of acti-

[1] E. M. KOSOWER and N. S. KOSOWER, Nature *224*, 117 (1969).
[2] N. S. KOSOWER, B. WERTHEIM, E. M. KOSOWER and W. CORREA, Biochem. biophys. Res. Commun. *37*, 593 (1969).
[3] H. M. LENHOFF, E. M. KOSOWER and N. S. KOSOWER, Nature *224*, 717 (1969).
[4] N. S. KOSOWER, K. R. SONG and E. M. KOSOWER, Biochim. biophys. Acta *192*, 1, 15, 23 (1969).
[5] N. S. KOSOWER, K. R. SONG, E. M. KOSOWER and W. CORREA, Biochim. biophys. Acta *192*, 8 (1969).
[6] R. GALUN, E. M. KOSOWER and N. S. KOSOWER, Nature *224*, 181 (1969).
[7] D. YAFFE, in *Current Topics in Developmental Biology* (Academic Press, New York 1969), vol. 4, in press.

vity within 20–60 sec. (The times are upper limits because of the need to scan the entire plate.) The replacement of the medium with fresh medium did not cause an immediate resumption of activity, but all cultures showed weak fibre contractile activity after 70–80 min at 37 °C. Full strong activity was observed after 4 h at 37 °C. No other changes were noted on further incubation.

Lower concentrations of diamide ($1–2 \times 10^{-4} M$) were without appreciable effect upon the contractile activity of the fibres in the cultures. Higher concentrations ($3 \times 10^{-3} M$) of diamide caused an instantaneous loss of contractile activity, observed through the microscope during the addition of the reagent.

The specificity of our thiol-oxidizing agents has been detailed elsewhere[1,2,5]. The rapid cessation of contractile activity in muscle fibres after intracellular oxidation of glutathione to the disulphide implies a close and possibly direct role for GSH in the contractile activity. Further support for this role is found in the length of the time for recovery of contractile activity, a time reasonable for the intracellular regeneration of GSH from GSSG[2]. The spontaneous contractile activity of muscle fibres in culture may bear a close relationship to the acetylcholine stimulated activity in denervated muscles[8,9]. The molecular basis for the participation of GSH in muscle contraction is under investigation.

Résumé. Le traitement de fibres en pulsation d'un muscle (cultivé en vase clos) avec l'oxydant diamide, spécifique pour la conversion du glutathione (GSH) au disulfide (GSSG), arrête vite tout mouvement. Après quelques heures d'incubation, le niveau normal de l'activité est retabli.

E. M. KOSOWER[10,11]

Department of Biophysics,
Weizmann Institute of Science,
Rehovot (Israel), 13 January 1970.

[8] S. THESLEFF, Ann. N.Y. Acad. Sci. *94*, 535 (1961).
[9] H. GRUNDFEST, in *Essays on Physiological Evolution* (Ed. J. W. S. PRINDLE; Pergamon Press, Oxford 1965), p. 119.
[10] National Science Foundation Senior Postdoctoral Fellow 1968/69. Permanent address: Dept. of Chemistry, State University of New York at Stony Brook, Stony Brook (New York, USA 11790).
[11] The author is grateful to Dr. DAVID YAFFE for advice and encouragement.

Effects of Cyclohexylamine on Rat Fertility

The biological effects of cyclohexylamine, the major degradation product of cyclamate, on chromosomes, reproduction and teratogenesis have been the subject of several papers[1–4]. In an earlier communication, a deleterious influence of cyclohexylamine sulphate on male fertility was described[3]. Later investigations showed that females mated with cyclohexylamine sulphate-treated males had smaller litters at term than those bred with controls. The finding was suggestive of genetic damage and forms the subject of this report.

Male Wistar albino rats weighing 175–200 g were randomly assigned to test (15 animals) and control (10 animals) groups. The test males were fed 0.2% cyclohexylamine sulphate (CHS) in their drinking water. At the same time, a 65-day breeding program was initiated in which each male was isolated with 2 virgin females for 5 days for a total of 13 sequential mating trials for each of the 25 males. During the first 3 mating trials, the females as well as the males drank the test solution at an average daily rate of 142 mg/kg body weight. After 3 trials, the mode of CHS administration was changed to gavage (220 mg/kg/day) so that the treatment was restricted to the males. Control males received distilled water by gavage. After another 4 mating trials, CHS treatment was suspended for the duration of 3 trials. During the final 2 breeding trials, CHS treatment by gavage (220 mg/kg/day) was re-instituted in the males.

Fifteen days after being separated from the males, the females were sacrificed to ascertain pregnancies, and to record the numbers of viable and nonviable embryos and resorption sites. The fetuses were examined for external defects and skeletal malformations.

Male fertility was calculated as the number of females impregnated relative to the number exposed. The male fertility and implantations (viable and nonviable embryos and resorption sites) data were analyzed using the Sign Test for time effects and the Wilcoxon–Mann–Whitney ranking test for treated:control comparisons[5].

Fertility in the treated group was generally impaired relative to the control group ($P < 0.05$ by one-tailed test). Fertility is plotted in Figure A. The adverse effect was apparently related to CHS dosing of males since the effect continued after treatment of females was halted. Observation on mating behaviour revealed no reduction in male or female libido. The antifertility effect persisted during the 3 trials in which the CHS treatment was suspended, indicating the effect to be of more than transitory nature.

The incidences of resorption sites and nonviable embryos in the test and control groups were similar in all trials, which excludes the possibility of a postimplantation embryocidal effect associated with CHS. However, the average number of implantations per litter was consistently and significantly ($P < 0.01$ by one-tailed test) decreased in the treated group. Data on viable and nonviable embryos and resorption sites were combined to compare postimplantational reproductive efficiency in both groups (Figure B). The depression in numbers of implantations could be accounted for by preimplantational loss apparently due to CHS treatment. In a previous study[3] in which the maximum dose of CHS was lower than that reported here, interference with embryonal viability at pre- and postimplantation stages was not observed; however, the antifertility effect of CHS in males was observed at as small a dose as 22.26 mg/kg/day as measured by ability to induce pregnancy.

Since the incidence and types of external defects and skeletal anomalies in embryos obtained from test animals were not different from the controls, the possibility

[1] M. S. LEGATOR, K. A. PALMER, S. GREEN and K. W. PETERSON, Science *165*, 1139 (1969).
[2] G. L. KENNEDY, P. G. SANDERS, M. S. WEINBERG, D. W. ARNOLD and M. L. KEPLINGER, Toxic. appl. Pharmac. *14*, 656 (1969).
[3] K. S. KHERA, D. R. STOLTZ, S. W. GUNNER, D. A. LYON and H. C. GRICE, to be published (1970).
[4] D. R. STOLTZ, K. S. KHERA, R. BENDALL and S. W. GUNNER, Science *167*, 1501 (1970).
[5] S. SIEGEL, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill Co. Inc., New York 1956).