Tabelle I. Labor- und Gewächshausversuche.

Produkt (Aufarbeitung)	Bonitierte Wirkung					
	Objektträger-test 1–0,01% AS	im Sellericblatt-Test			im Reben-Test	
		0,25	0,1	0,01% AS	präventiv 0,125–0,025%	kurativ 0,05–0,025%
Chlorsulfan	6,4 a)	98,5	85	58	100	14,6
Norsulfan	4,9	100	35	25	100	10,4
Mesulfan	6,5	100	100	67	100	0
Captan	6,1	100	95	80	100	12,5
Captan (50 WP) b)		100	100	89		
Zineb (65 WP) c)		100	79	69		

a) 0 = keine Wirkung, 9 = maximale Wirkung;
b) Common name für N-Trichlormethylthiotetrahydrophtalimid. Anw. Konz. 2, 0,4, 0,08%.
c) Common name für Zink-äthylen-bis-dithiocarbamat. Anw. Konz. 2, 0,4, 0,8%.

Tabelle II. Freilandversuche.

Produkt	durchschnittlich bonitierte Wirkung	
	Reben (*Plasmopara viticola*)	Apfelbaum (*Venturia inaequalis*)
Chlorsulfan a)	68–98	64–78
Norsulfan a)	41–97	70–80
Mesulfan a)	76–99	88–94
Captan a)	56–99	86–94
Bordeauxbrühe b)	95	
Kupfer-Zineb Kombination c)	97	
Kupferoxychlorid + Ultraschwefel c)		70–87
Glyodin-Präparat c)		75

a) aufgearbeitet zu 50% «wettable powder»; 125 g AS auf 100 l Brühe; b) Anwendung 1%;
c) Anwendungskonzentration nach Angabe des Herstellers.

Sowohl die Gewächshaus- wie die Freilandversuche zeigten, dass die 3 Substanzen an den behandelten Pflanzen keine phytotoxischen Nebenerscheinungen bewirken, sondern im Gegenteil das Wachstum und die Fruchtentwicklung günstig beeinflussen.

Das fungizide Wirkungsspektrum der 3 Substanzen scheint sich zu decken und ist nicht so breit wie das anorganischer Fungizide, vor allem der Cu-Präparate. Wie die Freilandversuche zeigen, kommen diese Präparate zur Bekämpfung der wichtigsten Pilzkrankheiten im Weinbau (*Plasmopara viticola*)[1] und Obstbau (*Venturia inaequalis*) in Frage. Gegen *Phytophthora infestans* auf Kartoffeln und *Septoria apii* übten sie eine gewisse Wirkung aus; bei der unter europäischen Verhältnissen üblichen Spritzfolge scheint die Dauerwirkung jedoch gegen die beiden letztgenannten Pilze ungenügend zu sein. Die übrigen Anwendungsgebiete müssen durch Feldversuche weiter abgeklärt werden[2].

Von den 3 Präparaten scheint nach den bisher vorliegenden Versuchsergebnissen Mesulfan am interessantesten zu sein.

R. WAEFFLER, R. GASSER,
A. MARGOT und H. GYSIN

Aus den Forschungslaboratorien der J. R. Geigy AG., Basel, den 18. April 1955.

[1] D. BOUBALS, A. VERGNES und H. BOBO konnten unsere Resultate mit Chlorsulfan bestätigen. (Essais de Fongicides organiques dans la lutte contre le mildiou de la vigne effectués en 1954. Progr. agric. vitic., Montpellier 1955.)

[2] Über Untersuchungen in den USA., die unsere Ergebnisse bestätigen, wird Mc NEW in den Contr. Boyce Thompson Inst. berichten.

Summary

A new group of organic compounds was tested with several laboratory methods, in the green-house and against different fungus diseases in the field. According to these tests the described sulfonamide derivatives show a fungicidal activity comparable to compounds which are actually used. So far Mesulfane seems to be the most promising compound.

Enzyme Levels in the Growing and Regressing Flexner-Jobling Carcinoma

Little is known about the enzymic pattern of spontaneously regressing tumors as compared to the enzyme levels in tumors still increasing in growth. Such a study seemed to us all the more interesting since it might eventually offer a basis of comparison for the enzymic changes occurring in tumors following *induced* regression by various therapies or radiation. The Flexner-Jobling rat carcinoma[1] having regularly a 35 to 40 per cent rate of spontaneous regression proved extremely suitable for this investigation. Generally speaking, once this tumor has been implanted into the subcutaneous tissue of the rat, its course may follow any of the following three directions:

(1) It may grow to a maximal weight of about 35 g finally causing the death of its host.

[1] K. SUGIURA and C. S. H. STOCK, Cancer *5*, 382 (1952).

(2) It may cause necrosis of and break through the skin at an earlier stage, between the 15th and 30th day after implantation. In this case the rats usually recover. Deaths from secondary infections are rare. The tumor will disintegrate by necrosis and be replaced by scar tissue.

(3) Regression of the tumor may start any time between the 14th and 30th day.

In the present study we have focused our attention on three enzymic activities: Catheptic activity, acid phosphatase and alkaline phosphatase. Catheptic activity has been estimated by the liberation of tyrosin from hemoglobin[1] while phosphatase activity was calculated from the amount of phenol liberated from phenylphosphate[2]. In both instances, the modified Folin method of LOWRY and his associates[3] was used, employing in the first a tyrosin standard and in the latter a phenol standard curve[4]. Representative assay systems are given at the foot of Table I and II.

The areas of the tumors were measured with calipers at three days' intervals. This method made it inevitable that by the time a tumor had been clearly recognized as regressing, it had lost already a substantial part of its former tissue. The values given, therefore, refer to tumors already well past their initial regression stage. After excision from the rat carcass they were washed in isotonic ice-cold KCl, dissected and, as far as possible, the non-necrotic tissue, usually the outer layer, was used.

As shown in Table I there is a sharp rise in catheptic activity in the regressing tumors as compared to the non-regressing ones. However, in neither group is there a direct relationship between tumor size and enzyme activity. We have, however, given a breakdown of the activities in each group for the better understanding of their distribution. The remarkable increase in catheptic activity in the course of regression may be an intrinsic part of a process, an "autolysis *in vivo*" by which the tumor protein is broken down and removed. It clearly emerges from the data presented that the highest activities observed in non-regressing tumors are well below the lowest activities in the regressing ones.

The results with the phosphatases are summarized in Table II. Whereas the acid phosphatase is not subjected to any significant changes, it is the activity of the alkaline phosphatase which is markedly decreased in regressing tumors; there is also evident a tendency towards lower alkaline phosphatase activity in the initial stage of tumor growth. We have expressed those changes as the phosphatase ratio (column 4). In non-regressing tumors past their ninth day of growth there was only one instance when it was as low as 0·9; in regressing tumors, on the other hand, the highest value obtained was 0·6, also in one instance only. It is noteworthy that in tumors starting their regression between the 14th and the 20th day (the minority) the above ratio was found to be 0·4 on the average, whereas it averaged 0·18 in tumors regressing between the 20th and 30th day. (In the Table the tumor activities have been grouped according to their ranges and not according to the age of the tumors.)

Alkaline phosphatase in regressing tumors thus seems to go through a maximum: It is low in the initial stage, increases with the growth of the tumor and then again decreases to its lowest activity once the tumor regresses.

[1] M. E. MAVER, A. E. GRECO, E. LOVTRUP, and A. J. DALTON, J. Nat. Cancer Inst. *13*, 687 (1952).

[2] R. IWATZURU, Bioch. Z. *173*, 384 (1926).

[3] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL, J. Biol. Chem. *193*, 265 (1951).

[4] W. KOPPERHAAR, Z. Anal. Chem. *15*, 233 (1876).

Table I.–Average Catheptic Activities.

Number of tumors	Activity	Weight range in grams	Description of implant
20	220	0·61 –30	Growing tumors
13	336	0·23 – 6·8	between the 6th and
3	*435*	0·98 – 8·5	the 38th day
Average of 36	276		
7	1613	0·040– 1·6	Regressing tumors
12	1194	0·070– 0·96	between 15th and
9	*748*	0·014– 1·12	38th day
Average of 28	1136		

Activities are expressed as micrograms of tyrosine liberated in one h per mg of homogenate-N. The assay system contained in 5 ml of 0·1 M acetate buffer, pH 3·5, hemoglobin corresponding to 18 mg of protein-N (approximately a 2·5 per cent solution of hemoglobin) to which was added 1 ml of tumor homogenate corresponding to amounts ranging between 2·5 and 0·8 mg of homogenate-N. (Enzymatic activity in this interval is linear.) Incubated for 30 minutes at 37°C. Incubation terminated by addition of 1 ml of 49 per cent trichloroacetic acid.

Table II.–Alkaline and Acid Phosphatase Activity.

Number of tumors	pH 4·5	pH 9·4	Phosphatase ratio*	Description of implants
1	475	92	0·19	Growing tumors
9	459	283	0·61	between 6th and
2	*524*	*508*	*0·96*	7th day. Range of
Average of 12	474	304	0·64	weight: 0·18–0·89 g
9	472	464	0·98	Growing tumors
10	434	627	1·44	between 9th and
6	*346*	*785*	*2·26*	38th day. Range of
Average of 25	426	606	1·42	weight: 1·02–30 g
4	628	27	0·04	Regressing tumors
9	581	153	0·26	between 15th and
5	*555*	*298*	*0·53*	38th day. Range of
Average of 18	584	165	0·28	weight: 0·14–1·60 g

* Expressed as alkaline phosphatase activity or acid phosphatase activity. Activities are expressed in micrograms of phenol liberated from phenylphosphate per milligram of tumor homogenate-N in one hour. The assay systems were as follows:

(a) *Acid phosphatase.* 5 ml of 0·1 M acetate buffer, pH 4·5, contained 2·181 mg of phenylphosphate. 1 ml of tumor homogenate was added, corresponding to amounts ranging from 0·9–1·2 mg of homogenate-N. Incubated 10 minutes at 37° C.

(b) *Alkaline phosphatase.* 5 ml of 0·025 M veronal buffer, pH 9·5, contained phenylphosphate as above and 4·65 mg of $MgCl_2$. Homogenate concentration, time and temperature of incubation as in (a). The same homogenates were used for (a) and (b). In both instances incubation was terminated by the addition of 1 ml of 49 per cent trichloroacetic acid.

This study which has been in part supported by the U.S. Vitamin Corporation is now being extended to other enzyme systems and tumors[1].

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Department of Biochemistry, New York Medical College and Funk Foundation for Medical Research, New York, N.Y., December 22, 1954.

[1] C. FUNK, Bull. Funk Found. *1*, 3 (1953).

Zusammenfassung

Flexner-Jobling-Karzinome, die spontan regressieren, weisen eine zwei- bis vierfache Steigerung ihrer katheptischen Aktivität auf. Dabei ist die saure Phosphatase nicht veränderlich; die alkalische Phosphatase hingegen sinkt in regressierenden Tumoren auf einen Bruchteil ihrer ursprünglichen Aktivität.

Enzymic Activities of Vi Strains of *Salmonella typhosa* and their W Variants

SHRIVASTAVA *et al.*[1] sought to correlate the enzymatic activities of various strains of *S. typhosa* with the presence of Vi antigen in these strains. These authors reported marked differences in the oxidative metabolism of glutamic acid and tyrosine and concluded that the Vi antigen is in some way responsible for the higher metabolic activities of Vi containing strains. The strains examined by SHRIVASTAVA *et al.* represented the recognized possible antigenic combinations known to occur in *S. typhosa*. Thus, the Vi I strain contained predominantly Vi antigen, the Watson V strain possessed all three antigens, Vi, O, and H, the H901 strain contained the H and O antigens, while the O901 strain possessed O antigen only. Although these strains cover all antigenic variations of *S. typhosa*, their known antecedents clearly indicate that they are separate isolates and aside from possession of common antigens, they are unrelated. Consequently, the possibility must be considered that the observed variations in oxidative metabolism may be due to enzymatic differences inherent in each strain. If this were the case, such differences would not be correlated with the presence of the Vi antigen. In order to determine whether such a causal relationship actually exists, a number of V strains of *S. typhosa* were compared with W variants (substrains) isolated directly from each of the strains. In one instance a V form substrain isolated from strain H901 following mouse passage was compared with its original non-Vi strain[2].

The strains examined in these experiments are given in the table. The cells were grown on meat extract agar plates for 18 h at 37°C, and were harvested, washed and resuspended in M/15 phosphate buffer. The suspensions were then adjusted to a final density of 500 as measured with the Klett-Summerson photoelectric colorimeter using the blue filter (420 mμ). Oxidative activities were followed manometrically at 37°C in the usual Warburg apparatus. Final volume in all vessels was 2·5 ml, which included 2·0 ml cells in the main compartment, 0·4 ml substrate in the side arm (either M/50 l-glutamic acid, M/50 tyrosine or 20% glucose), and 0·1 ml 20% KOH in center well.

The results of this comparison of activities on the substrates tested indicated identical uptake by V and W forms of the same strain. Thus, the data obtained for strain Ty2 V and Ty2 W when plotted as activity curves were found to be superimposed throughout the course of the experiment. Tests with the V and W forms of the Watson strain, the V and W forms of strain 58 as well as the V and W forms of strain H901 confirmed these results. The data clearly indicated that the V and W forms of each strain behaved in an identical manner

Oxidation of Glutamic Acid by V and W Substrains

S. typhosa Strain	$\mu l\ O_2$	
	60 min	120 min
Ty2 V	164	347
Ty2 W	170	355
Watson V	104	214
Watson W	102	208
H901 V	130	264
H901 W	124	258
58 V	74	158
58 W	82	176

towards the substrates tested (glucose, tyrosine and glutamic acid). However, it will be seen that differences existed between the strains. These experiments demonstrate that the interstrain differences cannot be correlated with the presence or absence of the Vi antigen.

FELIX and PITT[1] have established the important role played by the Vi antigen in mouse virulence. The nutritional requirements of the culture as a major factor in mouse virulence have been clarified by BACON *et al.*[2], and FORMAL *et al.*[3]. Any attempt to correlate enzymatic activities of the various typhoid strains with antigenicity and virulence should take into account the individual differences of strains having different origins. Thus, any comparison of Vi and non-Vi containing strains for effects attributable to the Vi component is best accomplished by the use of W variants isolated from the same Vi strain. This is further borne out in a later paper by the authors themselves in their study of aryl sulphatase activity in *S. typhosa*[4]. The fact that only strain Vi I demonstrated any appreciable activity while other Vi strains tested were essentially inactive further serves to emphasize the desirability of including the W form of strain Vi I as a control[5].

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Zusammenfassung

Enzymatische Aktivitäten in V-Stämmen von *Salmonella typhosa* wurden mit solchen in W-Stämmen, welche aus V-Kulturen isoliert worden waren, verglichen. Die Aktivitäten in V- und W-Formen gleicher Stämme waren identisch und sind daher unabhängig vom Vorhandensein oder Fehlen des Vi-Antigens.

[1] G. C. SHRIVASTAVA, S. C. AGARWALA, and S. S. BHATANAGAR, Exper. *9*, 421 (1953).

[2] L. S. BARON, unpublished data (1952).

[1] A. FELIX and R. M. PITT, J. Hyg. *49*, 92 (1951).

[2] G. A. BACON, T. W. BURROWS, and M. YATES, J. Exptl. Pathol. *32*, 85 (1951).

[3] S. B. FORMAL, L. S. BARON, and W. SPILMAN, J. Bacteriol. *68*, 117 (1954).

[4] G. C. SHRIVASTAVA, K. L. ARORA, and S. S. BHATANAGAR, Exper. *10*, 493 (1954).

[5] G. P. GLADSTONE, Br. J. Exptl. Path. *18*, 67 (1937). Growth of Vi strains in synthetic media where amino acids provide the sole carbon source results in rapid conversion to the non-Vi state. This provides a convenient means of obtaining W substrains.