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Biochemical Changes in Mouse Lung Concomitant with Influenza Virus Infection.* (21051)

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(Introduced by A. F. Rasmussen, Jr.)
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In an attempt to gain information concerning the metabolic interrelationships between the host cells of mouse lung and the invasion and proliferation of influenza virus, studies of the *in vitro* metabolism of both normal and of Type A, PR8 influenza virus infected mouse lung were carried out. The enzymic activities of normal and virus infected lung tissue homogenates were compared by measuring the oxygen uptakes consequent upon the addition of various substrates. A stimulated or characteristically altered metabolic rate in infected tissue might reflect a diversion of normal host metabolism toward viral synthesis. Accordingly, compounds generally

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associated with providing energy for the synthesis of protoplasm and compounds assumed to be important in nucleic acid metabolism were investigated. Since it was found that the activity of xanthine oxidase was increased 2 to 2.5 times in the infected tissue, experiments were carried out to determine if this activity was due to an increase in the level of the same kind of enzyme in the tissue, or, if the presence of the proliferating virus brought about a difference in kind of enzyme. The effect produced by several enzyme poisons on the activity of xanthine oxidase in normal lung was compared with that of the poison-effect on the enzyme activity in infected lung. The influence of methylene blue on the 2 systems was investigated, presumably, to detect possible differences between the "dehydrogenase" and "oxidase" parts of the enzyme molecule(1). Assays for possible end-products of hypoxanthine-xanthine oxidations were made and the results compared.

These experiments were essentially exploratory in nature, intended to initiate a series of experiments designed to elucidate the role of host tissue metabolism in the process of influenza virus proliferation.

Methods and materials. The Type A, PR8 strain of influenza virus used throughout these investigations was obtained originally from Dr. Carl J. DeBoer at Birmingham Veterans Hospital, Van Nuys, Calif. During the course of this work, the virus has been through 63 mouse lung passages and was sufficiently pathogenic for 4- to 6-week-old albino Swiss mice weighing from 10 to 16 g so that the intranasal inoculation of up to a 10^{-4} dilution led to a fatal disease associated with extensive pneumonia. The animals were infected intranasally under light ether anesthesia, with an inoculum of 0.03 ml of a 10^{-3} dilution of infected lung tissue (10 to 100 lethal doses). Lung tissue homogenate from paired normal and 48- to 72-hour infected mice was used as a source of enzyme. The mice were killed by a sharp blow to the head, the lungs excised and placed on a moistened piece of sterile filter paper contained in a petri-dish packed in crushed ice. The tissue was trimmed, blotted free of excess blood and ground in 4 ml ice-cold phosphate buffer at pH 7.4 in the type of homogenizer developed by Brendler(2). The lungs of 6 mice were pooled for each experiment excepting where differences between individual animals were under investigation. The presence of virus was established by animal inoculation and by the hemagglutination method of Hirst as modified by Salk(3). The enzymic activity of the homogenate was determined manometrically by means of a conventional Warburg apparatus, xanthine oxidase according to the method of Axelrod and Elvehjem(4) and the effect of methylene blue on the rate of oxygen consumption in the text mixtures according to Rickert and Westerfeld(1). The difference between the respiration of the homogenate in the presence and absence of substrate was calculated and recorded as activity produced by a given substrate. All Warburg flasks used in the determinations contained 1.5 ml of homogenate (equivalent on an average, to 15 mg dry weight) in a total fluid volume of 3.2 ml at a pH of 7.4 and containing 250 μg of streptomycin plus 1500 units of penicillin. After many of the Warburg experiments assays for certain end-products were made on the flask contents; uric acid determinations were made according to the method of Folin as modified by Heplar and Stoskope(5); urea determinations by the method of Karr as described by Hawk *et al.*(6); and ammonia determinations by the method of Van Slyke and Cullen described by Hawk *et al.*(6). The inhibition of xanthine oxidase by tetraethylthiuram disulphide (Antabuse) was investigated by the method of Rickert *et al.*(7) and that of 2-amino-4-hydroxy-6-pteridinecarbonzaldehyde (6-pteridylaldehyde) according to Kalcar as described by Van Meter and Oleson(8).

Results. Results of experiments with compounds, the oxidative decomposition of which may give rise to energy yielding mechanisms, are presented in Table I. The substrates were used as their sodium salts where feasible, 10 μM in 0.2 ml volume being used routinely. The values recorded are the average of at least 3 experiments and represent the oxygen uptake of 20 mg tissue (dry weight equivalent) per 20 minutes stimulated by the addition of substrate. In each experiment the

TABLE I. Oxygen Consumption of Normal and of PR8 Influenza Virus Infected Mouse Lung upon Addition of Compounds Capable of Yielding Energy.

Compound tested	μl O_2 uptake/20 min./20 mg dry lung	
	Normal lung	Infected lung
Formate	1	1
Acetate	2	2
Lactate	2	6
Citrate	0	0
Cis-aconate	1	2
Alpha-ketoglutarate	4	4
Succinate	16	12
Fumarate	4	2
Maleate	0	0
Malate	0	3
Malonate	3	3
Pyruvate	2	2
Oxalacetate	1	0
Ribose	0	0
Glucose	1	1
Fructose-di-phosphate	0	0
Alpha-glycerophosphate	1	3
Glycerol	1	2
Benzoate	1	3
Glutamate	0	0
Aspartate	0	0
l-Histidine	0	0
l-Proline	0	0
l-Alanine	0	0
dl-Serine	2	0
Glycine	0	0

oxygen uptake representing the endogenous respiration was subtracted from the reported values. In spite of the fact that there are some differences between the values obtained for the 2 different homogenates, the total over-all values are of such low order as to make the significance of these figures doubtful. Of particular interest was the finding that, on a comparative basis, succinate produced the greatest stimulation in oxygen uptake in both normal and infected tissue and that this stimulation was greater in normal tissue. Ackermann(9) has found that the multiplication of influenza virus is inhibited in chick embryo chorioallantoic tissue cultures by malonic acid and by Antimycin A; both succinic dehydrogenase inhibitors. If succinate oxidation contributes to the over-all process of influenza virus multiplication, then this enzymic reaction is readily available in mouse lung.

The results of experiments with compounds related to nucleic acid metabolism are presented in Table II. The purine and pyrimidine bases as well as the nucleosides were employed at a final concentration of 10 μM per flask, the nucleotides at 5 μM and the thymus nucleic acid at 2.5 μM per flask. It is apparent that any demonstrable activity in normal lung was increased 2 to 2.5 times in the virus infected tissue. In some cases actual decrease in oxygen consumption was observed, *i.e.*, the oxygen uptake was less in the flasks with substrate than in the endogenous flasks. The addition of adenine generally inhibited oxygen uptake as did the addition of orotic acid. The inhibition caused by adenine provides for interesting speculation when one considers that adenine is a part of the xanthine oxidase molecule. It is believed that the oxygen uptake was, in a large measure, due to xanthine oxidase activity since the other steps, so far as is known in the breakdown of these compounds, are hydrolytic rather than oxidative(10). Most likely the compounds were degraded to hypoxanthine or xanthine and thence oxidized to uric acid. In order to test this hypothesis, assays for uric acid(5) were made on the flask contents of

TABLE II. Enzymic Activity of Purines and Pyrimidines and Other Compounds Related to Nucleic Acid Metabolism in Normal and in PR8 Influenza Infected Mouse Lung.

Compound tested	μl O_2/20 min./20 mg dry lung*	
	Normal lung	Infected lung
Hypoxanthine	6	13
Inosine	6	12
Xanthine	4	10
Xanthosine	3	7
Adenine†	0	0
Adenosine	5	12
Adenylic acid	2	6
Guanine	0	3
Guanosine	1	4
Guanylic acid	0	2
Uric acid	0	0
Cytosine	0	0
Cytidine	0	0
Cytidylic acid	0	0
Uracil	0	0
Uridylic acid	0	0
Thymine	0	0
Orotic acid†	0	0
Thymus nucleic acid	0	0

* Recorded values are average of at least 3 experiments and calculated after endogenous oxygen uptake had been subtracted.

† O_2 uptake was actually lower than endogenous upon addition of these substrates.

TABLE III. Influence of Methylene Blue (M.B.) on Oxygen Uptake Stimulated by Purine Compounds Showing Enzymic Activity and Resultant Accumulation of Uric Acid.

Compound tested	Normal lung				Infected lung			
	O_2 uptake*		Uric acid†		O_2 uptake		Uric acid	
	—	+M.B.‡	—	+M.B.	—	+M.B.	—	+M.B.
Hypoxanthine	7	14	0	.03	13	21	.03	.24
Inosine	6	12	0	.14	11	19	.18	.30
Xanthine	5	9	.03	.36	10	16	.36	.45
Xanthosine	4	6	.02	.09	7	11	.10	.34
Adenosine	5	9	0	.11	12	17	.12	.20
Adenylic acid	2	4	0	.03	6	9	.06	.14
Guanine	1	3	0	.07	4	6	.03	.09

* μl/20 min./20 mg dry lung.
† Mg accumulating/ml of flask content at end of 100 min. incubation.
‡ Methylene blue, 0.2 ml of 0.0113 M solution was added as a part of the 3.2 ml flask fluid volume.

the flasks showing an increase in oxygen consumption. A protein-free filtrate was prepared and treated with a specific uric acid reagent in the presence of optimal amounts of urea and sodium cyanide. The blue color produced by the reduction of alkaline phosphotungstate by urate was determined in a photoelectric colorimeter. In addition, since it has been demonstrated that the addition of methylene blue to such a test-system increases the activity of xanthine oxidase(1), an investigation was made of the influence of the dye on oxygen consumption stimulated by addition of these substrates and additional assays for uric acid made on the flask contents. The results of these experiments are recorded in Table III. In the flasks showing maximal oxygen uptake there was also an increase in the amount of uric acid formed. Also, where there was enzymic activity, the addition of methylene blue increased this activity. Assays for ammonia(6) carried out by treating the flask contents with an equal volume of saturated carbonate solution and transferring the liberated ammonia by distillation into a boric acid receiving solution where it was titrated directly with a standard acid solution, revealed that in the flasks showing maximal oxygen uptake there was a small increase in the amount of ammonia produced; from 0.2 to 3.3 mM per ml of flask content. Uricase was not found in mouse lung.

Results of the experiments recorded in Table IV provide additional evidence that the activity of xanthine oxidase is increased in infected lung. It can be seen that methylene blue apparently increased the enzymic activity in both normal and infected tissue; the addition of dye did not elicit a differential effect. Table V indicates the effect of cyanide on the xanthine oxidase found in virus infected lung and the results of attempts to reverse this effect with either cytochrome *c* or ferrous sulphate. In the flasks without methylene blue, the inhibition was irreversible, *i.e.*, by either cytochrome *c* or ferrous ions. In flasks containing methylene blue the inhibition was partially reversed by either cytochrome *c* or ferrous sulphate. This does not necessarily indicate that the xanthine oxidase is linked to

TABLE IV. Influence of Methylene Blue (M.B.) on Xanthine Oxidase Activity in 6 Normal and 6 Influenza Virus Infected Mouse Lung Homogenates.

	μl oxygen consumed/20 min./20 mg dry lung					
	Xanthine		Hypoxanthine		O_2 increase in M.B.	
	−M.B.	+M.B.	−M.B.	+M.B.	Xant.	Hypo-xant.
	Infected lungs					
	5.5	10.8	8.8	22.6	5.3	13.8
	5.8	21.2	10.0	20.2	15.4	10.2
	11.1	29.0	12.6	28.1	17.9	15.5
	12.2	30.4	14.4	33.4	18.2	29.0
	14.1	27.1	14.6	27.9	13.0	13.3
	15.5	29.3	19.7	37.0	13.8	17.3
Avg	10.7	24.6	13.3	28.2	14.3	14.9
	Normal lungs					
	2.3	10.4	2.8	9.3	8.1	6.5
	3.6	16.2	4.0	10.4	12.6	6.4
	3.9	24.6	3.8	19.6	20.7	15.8
	4.2	9.5	5.3	13.9	5.3	8.6
	5.1	16.8	5.0	15.0	11.7	10.0
	5.9	11.1	6.5	11.6	5.2	5.1
Avg	4.2	14.8	4.6	13.3	10.6	8.7

TABLE V. Effect of HCN on Xanthine Oxidase Activity in Mouse Lung Infected with PR8 Influenza Virus and Reversal of This Effect by Cytochrome *C* and by Ferrous Ions.

Compounds tested*	μl O_2 uptake/20 min./20 mg dry lung –M.B.	% inhibition	+M.B.	% inhibition
Controls	10.6		22.6	
10^{-3} M HCN*	1.3	88	3.0	87
Idem + 10^{-3} M cytochrome *c*†	1.6	85	9.0	61
Idem + 10^{-3} M $FeSO_4$†	1.3	88	8.0	65

* The correct mixtures of KOH-KCN were employed in the center-wells to give this concentration of HCN in the flasks.

† Concentrations are number of moles/ml of final fluid volume.

the cytochrome system since the same result can be obtained by substituting ferrous sulphate for the cytochrome, however, it has been shown that cytochrome *c* can be reduced by xanthine oxidase(11,12). The results of experiments with other inhibitors, *i.e.*, azide, arsenite, monoiodoacetate and monofluoroacetate are tabulated in Table VI. Azide, in the lowest dilution used, inhibited the oxygen uptake by 40%; in the presence of methylene blue this inhibition was reduced in 12%. The highest dilution of azide produced a similar, although greatly diminished, effect. Likewise, in the absence of methylene blue, arsenite at 0.0005 M concentration produced 89 and 73% inhibition respectively; 0.0005 M concentration produced 89 to 61% inhibition. Iodoacetate in 0.01 M concentration reduced the activity of the xanthine oxidase by 100% when methylene blue was not present; addition of dye reduced this value by one-half. Fluoroacetate, by comparison, was not a potent inhibitor of this system either in the presence or absence of methylene blue, in fact, it produced a 13% stimulation in oxygen uptake in the homogenate to which the dye had been added. Antabuse did not prove to be a potent inhibitor in this system. On the other hand, 6-pteridyl aldehyde at optimal concentrations completely inhibited the enzyme and the addition of methylene blue did not alter the result, thus indicating action at the substrate level and the prevention of hypoxanthine and xanthine activation. Since the results with these inhibitors on the enzyme found in infected tissue were similar to the results with normal tissue, details of the experiments with the latter will not be reported here.

Assays were made for uric acid, ammonia, and urea accumulating either directly or indirectly from xanthine oxidase activity. When hypoxanthine was added as substrate the amount of uric acid accumulating per Warburg flask after 100 minutes incubation ranged from zero to 0.30 mg per flask. If xanthine were the added substrate then the resulting uric acid values ranged from 0.03 to 0.54 mg per flask. The addition of methylene blue substantially increased the amount of uric acid formed; this effect being more marked when hypoxanthine was the added substrate. The accumulation of uric acid revealed no qualitative difference in the xanthine oxidase activity; the enzyme from both normal and infected lung oxidized the hypoxanthine or xanthine to uric acid in proportion to the amount of enzyme present in the tissue. The results of ammonia assays on both normal and infected tissue failed to show an accumulation of the product resulting from the enzymic degradation of hypoxanthine or xanthine. Assays for urea production(6), made by converting the urea in protein-free filtrates to ammonium car-

TABLE VI. Effects of Sodium Azide, Sodium Arsenite, Sodium Monoiodoacetate and Sodium Monofluoroacetate on Xanthine Oxidase Activity of Mouse Lung Infected with PR8 Influenza Virus.

Compounds tested*	μl O_2 uptake/20 min./20 mg dry lung –M.B.	% inhibition	+M.B.	% inhibition
Controls	14.2		33.0	
Azide M/ 2000	8.5	40	28.9	13
" M/10000	11.3	21	30.5	8
Arsenite M/ 200	.0	100	9.2	73
" M/2000	1.6	89	12.8	61
Controls	10.9		26.6	
Iodoacetate M/ 100	.0	100	13.3	50
" M/1000	9.2	16	24.6	8
Fluoroacetate M/100	9.3	16	23.4	10
" M/10000	11.6	0	30.1	0

* Inhibitor and homogenate were incubated for 20 min. before the substrate was tipped-in. Concentrations are number of moles/ml of final fluid volume.

TABLE VII. Correlation of Change in Virus Concentration with Change in Levels of Xanthine Oxidase Activity in Mouse Lung after Infection with PR8 Influenza Virus.

Time†	*Group 1 E.A.‡	H.T.§	U.A.‖	Group 2 E.A.	H.T.	U.A.	Group 3 E.A.	H.T.	U.A.	Average E.A.	H.T.	U.A.
0	1.0	0	.0	1.	0	.0	2.0	0	.0	1.3	0	.0
24	2.3	80	.06	8.	20	.18	2.0	20	.09	4.1	40	.11
48	3.0	80	.12	7.	640	.18	6.6	640	.18	5.5	453	.16
72	9.3	1280	.24	7.3	1280	.30	10.6	1280	.27	9.1	1280	.27
96	10.0	640	.30	7.3	40	.21	1.0	0	.09	4.8	226	.20

* Group of 5 mice, one chosen at random for examination at the indicated time.
† No. of hours between initial infection and titration.
‡ Enzyme activity: $\mu l\ O_2$/20 min./20 mg dry lung. (Xanthine was added as substrate.)
§ Hirst titer: the highest dilution of virus lung showing complete hemagglutination.
‖ Uric acid: total amount of compound per flask at end of experiment (100 min.) in mg.

bonate by the action of added urease, Nesslerizing in the presence of gum gatti and readings in a photoelectric colorimeter, did not show urea to be an end-product of the xanthine oxidase activity. Also, assays made on the unincubated homogenates failed to detect any difference between the *in vivo* urea content of normal and infected tissue.

An attempt was made to correlate the more quantitative aspects of virus multiplication with the increase in enzyme level; for purposes of convenience these were called "progression experiments." Results recorded in Table VII show a correlation between the day-by-day multiplication of virus in the mouse lung, as determined by the hemagglutination titer, and the change in enzyme level in the same lung, as determined by oxygen uptake and by uric acid production.

It was of interest to investigate the level of liver xanthine oxidase in mice concomitant with influenza virus infection. Therefore, liver and lung from the same animals were processed and examined in the same manner. While the lung enzyme from the infected mice showed a 2.5 fold interest in activity over that of the non-infected mice, the liver xanthine oxidase remained at the same level of activity as that of the non-infected animals.

Comment. The addition of any one of 26 compounds, the oxidation of which might possibly yield energy for the formation of influenza virus particles to normal and to virus infected mouse lung tissue homogenate revealed only minor enzymic differences between the 2 homogenates. Apparently, the propagation of influenza virus in mouse lung does not alter to a significant degree the level of host tissue enzymes concerned in the oxidation of these compounds. These experiments are in no sense complete since no attempt was made to follow in detail the reactions which were measured only by the rate of oxygen uptake. The question of energensis in the formation of virus particles is an elusive one. Certain information indicates that a functioning Krebs cycle is essential for the propagation of influenza virus both in mouse lung and chorioallantoic membrane tissue cultures(9,13). However, whether the parasite requires energy over and above that required for the normal cellular metabolic reactions of the host tissue remains to be established(14).

Since virus particles are composed of complex nucleoprotein material, the synthesis of which is stimulated by the entrance of virus particles into the host cell, it seems likely that at least a part of the increased *in vitro* activity in the virus infected lung caused by the addition of certain purine bases, nucleosides and nucleotides is indicative of special enzyme activity brought about by the presence of the viral infection. The greatest part of the oxygen uptake resulting from the metabolism of these compounds is probably due to xanthine oxidase activity as shown by the resultant accumulation of uric acid and ammonia and by the effect of methylene blue on the system. If this is true, then it serves as indirect evidence that certain purine deaminases, nucleosidases and nucleotidases are increased in the influenza virus infected mouse lung. Also, the importance of xanthine oxidase in the purine metabolism of mouse lung seems evident.

Although the results of the experiments re-

corded here clearly show that this infectious process is accompanied by an increase in the level of xanthine oxidase, the exact role of the enzyme remains to be determined. The "progression experiments" suggest that the enzymic activity is more than a reflection of an inflammatory process excited by the presence of virus in the tissue. In mice receiving 10 to 100 lethal doses of infecting virus, the increment and decrement periods in the level of xanthine oxidase activity roughly parallel the virus increment and decrement periods. Of possible significance here is the finding by Ackerman(15) that methionine is involved in the biosynthesis of influenza virus; the same amino acid is required in a diet favorable for the biosynthesis of xanthine oxidase(16,17). Bauer(18) reported a correlation between yellow fever infection and the development of xanthine oxidase in chick embryos. On the other hand, the role of hypoxanthine and xanthine in the purine metabolism of animals has not been considered important(19); however, more recent experiments tend to show that hypoxanthine may occupy a place of central importance in the metabolism of purine containing components in nucleic acids (20).

In an attempt to resolve the questions raised by the results of the experiments reported here, further experiments are contemplated to determine: (a) if non-specific inflammatory processes in mouse lung are accompanied by an elevation in the level of xanthine oxidase; (b) if the increased enzyme level indicates an increased breakdown of host nucleic acids, the end-products of which might be hypoxanthine and xanthine; and (c) if the action of xanthine oxidase is essential to the biosynthesis of influenza virus.

Summary. 1. Mouse lung tissue possesses the capacity to oxidize several of the naturally occurring compounds which are capable of undergoing oxidative decomposition and giving rise to energy-yielding mechanisms. Under the limiting conditions of these exploratory experiments, the presence of a Type A, PR8 influenza virus infection in this tissue does not appear to alter, to a significant degree, these oxidative reactions. 2. Influenza infection appears to increase the capacity of host lung tissue to metabolize several compounds concerned in nucleic acid metabolism. Whether this phenomenon is indicative of cellular breakdown incidental to the viral infectious process or whether it represents an "active" degradation of specific molecules consequent to virus synthesis is still to be determined. 3. Xanthine oxidase appears to play a central role in the *in vitro* purine metabolism of mouse lung; the activity of this enzyme is increased in the infected tissue. The increment and decrement periods of virus proliferation roughtly parallel the increment and decrement periods of enzyme activity. In no case was a qualitative difference found between the xanthine oxidases of normal and virus infected lung tissue; the effects produced by the addition of metabolic inhibitors, the influence of methylene blue, and the end-products of the reactions were the same. The significance of these findings has been discussed.

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A Non-Auxinic Growth-Promoting Factor Present in Crown Gall Tumor Tissue. (21052)

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An abundance of evidence published during the past decade has suggested that crown gall tumor cells proliferate abnormally because they elaborate in greater than regulatory amounts a growth-promoting substance(1-3). Although auxin synthesized by the tumor cell has commonly been regarded as playing an etiological role in abnormal growth of such cells, it is by no means certain that the characteristic behavior of the crown gall tumor cell can be explained solely or even in large part on the basis of high and unregulated auxin levels in those cells. An attempt was therefore made to determine whether, in addition to growth substances of the auxin type, non-auxinic factors capable of stimulating cell proliferation are present in plant tumor tissues.

Methods and materials. In order to investigate this problem it appeared desirable to use as a test object plant cell types that would not proliferate actively in White's culture medium containing auxin. Steward and Caplin(4) have recently reported that mature parenchymatous cells of the potato (*Solanum tuberosum* L.) are not stimulated to active cell division by 2,4-dichlorophenoxyacetic acid. These workers found, however, a synergistic effect to exist between 2,4-D and coconut milk in promoting cell division in the potato parenchyma tissue. Tobacco (*Nicotiana tabacum* L.) pith cells have been reported(5) to enlarge but not to divide under the influence of indole acetic acid. Tobacco pith tissue, which was used in this study, was isolated aseptically from the middle third of greenhouse-grown tobacco plants that were about 3 feet tall. Pith tissue fragments free of internal phloem were cultured either on White's basic medium containing 1% agar(6) or on that medium supplemented with the desired concentration of an auxin and/or tumor extract. The tumor extracts were obtained from sterile crown gall tissue grown in culture. The tumors were harvested during the period of active growth and placed in a Waring Blendor at 4°C. They were thoroughly macerated, pressed through cheesecloth, and centrifuged for 10 minutes at 7,000 r.p.m. The supernatant fluid was incorporated in White's medium in a concentration of 15% by volume in certain experiments described below. The pith tissues were cultured in 50 ml Erlenmeyer flasks containing a total of 14 ml of media. The cultures were maintained at room temperature in diffuse light.

Results. Tobacco pith tissue planted on White's basic medium showed a very limited or, in most instances, no wound healing response even after prolonged incubation, Fig. 1A. In the presence of suitable concentrations of growth substances of the auxin type, however, the volume of the pith tissue more than doubled in a 10-day period, Fig. 1B. The tissue frequently became contorted as if under considerable internal stress. Subsequently, the structure of the tissue itself showed a striking tendency to disintegrate. Enlarged pith cells projected in all directions from what was originally a compact, well organized tissue fragment. In accord with findings reported earlier(5) it was found that proliferation of the pith cells did not occur in the presence of the auxins used. In these experiments naphthalene acetic acid, indole butyric acid, indole acetic acid, and para-