# EFFECTS OF EXTRACTS OF NORMAL TISSUES AND OF TUMOURS ON YEAST FERMENTATION.

## ESTIMATIONS OF RELATIVE DIPHOSPHOPYRIDINE NUCLEOSIDASE ACTIVITIES

by

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HARPUR, JOHNSON AND QUASTEL[7] have found that extracts of brain and other tissues inhibit an actively fermenting yeast system. This result was explained in terms of destruction of DPN** by the tissue DPNase, and of competition for pyruvate by the tissue DPN-linked lactic dehydrogenase. It seemed to us that development of the system on a more quantitative basis might yield a convenient method for the estimation of the relative DPNase activities of normal and tumour tissues.

At pH 6.0, when the yeast carboxylase is fully active, it was found that under our experimental conditions the inhibition produced by a brain extract can be explained entirely in terms of DPNase (nucleosidase) activity, this inhibition of fermentation being almost completely antagonised by NAA. These observations have enabled us to develop a method of estimation of DPN-nucleosidase which eliminates the necessity for extraction and estimation of residual DPN. This procedure avoids possible inaccuracies introduced by incomplete extraction procedures as might be introduced, for example, by the presence of the "bound" form of DPN.

At pH 7.4 in a bicarbonate medium the yeast carboxylase is almost inactive, there being no gas evolution. Addition of a brain extract in the presence of NAA, however, gives rise to a rapid evolution of $CO_2$ due to the presence of a DPN-linked lactic dehydrogenase which supplies conditions for the removal of pyruvate and for the reoxidation of $DPNH_2$. This system was not studied in great detail.

The enzymic splitting of DPN by micro-organisms, plant and animal tissues has been known for many years and the work was reviewed by SCHLENK in 1945[22]. He concluded that "the immediate cause of inactivation of the nicotinamide nucleotides by animal tissues *in vitro* may be attributed to the presence of an enzyme capable of splitting nicotinamide from these substances". Since then, however, KORNBERG AND LINDBERG[11] and KORNBERG AND PRICER[12] have described a nucleotide pyrophosphatase degradation of DPN. MANN AND QUASTEL[14] had originally observed that relatively high

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** DPN = diphosphopyridine nucleotide; $DPNH_2$ = reduced diphosphopyridine nucleotide; HDP = hexose diphosphate; ATP = adenosine triphosphate; DPNase = diphosphopyridine nucleosidase; NAA = nicotinamide.

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concentrations of nicotinamide inhibit the inactivation of DPN by tissue preparations. It now appears that this compound specifically inhibits the nucleosidase, whilst having little or no effect on KORNBERG's pyrophosphatase.

HANDLER AND KLEIN[6] demonstrated the inactivation of pyridine nucleotides in preparations of brain, liver, kidney and muscle of rabbits, rats and dogs, and SPAULDING AND GRAHAM[24] have determined the relative activities of various normal tissues. MCILWAIN and his collaborators (MCILWAIN[15,16,17]; MCILWAIN AND RODNIGHT[19,20]; GORE, IBBOTT AND MCILWAIN[2]) have recently studied DPN breakdown in nervous tissue. No investigations have been reported on the estimation of DPNase in neoplasms although its presence has been demonstrated in tumour homogenates by LE PAGE[13] and NOVIKOFF, POTTER AND LE PAGE[21]. It is interesting to note that SCHLENK[23], BERNHEIM AND FELSOVANYI[1] and KENSLER, SUGIURA AND RHOADS[10] observed a reduction in the DPN content of tumours compared with normal tissues. WENNER, SPIRTES AND WEINHOUSE[25] in a study of the effect of added DPN on pyruvate oxidation by mouse tumour mitochondria have pointed out, in explanation of their results, that tumours may be very active in splitting DPN.

MCILWAIN[15] has stressed the remarkably high level of DPNase activity in brain tissue and has attempted to relate it to glycolytic activity (MCILWAIN[16,17]; GORE, IBBOTT AND MCILWAIN[2]).

## MATERIALS AND METHODS

*Yeast preparation.* An acetone-dried powder of yeast extract, prepared according to the method of HOCHSTER AND QUASTEL[8,9] was used throughout this investigation. A fresh aqueous suspension usually containing 60 mg/ml was prepared for each experiment.

*Tissue preparations. Acetone-dried beef brain.* Fresh beef brains, obtained from the abattoirs within an hour of killing, were dissected and the cortex freed as far as possible of white matter. After passing through a cold Latapie mincer, the mince was mixed with an equal volume of cold normal saline, thoroughly mixed, added to 10 vols cold acetone with vigorous stirring and filtered. After washing with cold acetone, the brain was transferred to a vacuum dessicator and dried over $CaCl_2$. The dry product was ground in a mortar and stored *in vacuo* at 4° C; in these circumstances the preparation retained its activity for at least 8 months.

For determination of DPNase activity, a weighed portion of the acetone powder was ground with distilled water in a cold mortar for 2 min. In some experiments the resulting suspension was used after making up to volume with water, whilst in others the supernatant was used after centrifuging at 1500 r.p.m. for 15 min and making up to volume.

*Tissue homogenates.* The tissues were quickly washed with water, dried on filter paper and a portion weighed into a Potter-Elvehjem all-glass homogeniser. The tougher tissues, *e.g.* muscle, heart and some tumours, were finely chopped with scissors before weighing and after such preliminary treatment yielded satisfactory homogenates. All tissues, including the human brain and other tumours were used within 30 to 60 min of excision, and all homogenates were made with water.

*Manometric methods.* Conventional Warburg manometers were used at 28° C. For experiments at pH 6.0, 0.02 *M* potassium phosphate buffer was used; at pH 7.4, 0.04 *M* $NaHCO_3$/7% $CO_2$ buffer. In all experiments the vessels were gassed with 93% $N_2$/7% $CO_2$ for 10 to 15 minutes.

## RESULTS

### *Fermentation system with yeast-powder at pH 6.0*

In order that the inhibitory effect of brain preparations on yeast fermentation might be placed on a more quantitative basis it was desirable to establish optimum conditions for the fermentation and to eliminate the initial lag period.

Initially the basic system contained 50 mg yeast powder, 0.03 *M* glucose and 0.02 *M* K-phosphate buffer, the glucose being tipped from the side-arm at zero time,

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(in all experiments, no gas exchange occurred until a substrate had been added). Addition of 0.005 *M* $MgCl_2$ and 0.001 *M* ATP increased the gas output to a maximal level but only after an initial lag period. However, it seemed likely that this delay in the outset of fermentation might be due to the absence, in the early stages, of a suitable H-acceptor for the re-oxidation of $DPNH_2$. The result obtained by the addition of pyruvate to the system indeed suggests that this is the case, as in these circumstances the lag period is almost completely abolished. 0.005 *M* pyruvate (tipped with the glucose from the side-arm) gave the maximal rate of gas output. These results are shown graphically in Fig. 1.

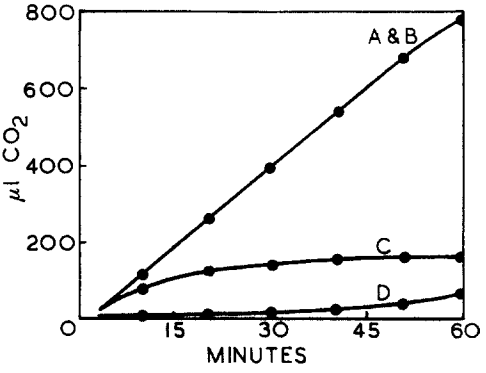


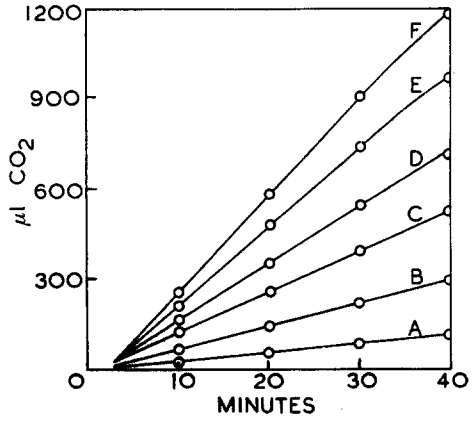

Fig. 1. Fermentation by yeast powder at pH 6.0. Each vessel contained 15 mg yeast; 0.02 *M* phosphate buffer pH 6.0; 0.005 *M* $MgCl_2$; 0.01 *M* NAA;. 0.001 *M* ATP. Glucose and pyruvate tipped at zero time from the side-arm. Total volume, 3.0 ml; temperature, 28° C; gas phase, $N_2$/7% $CO_2$.

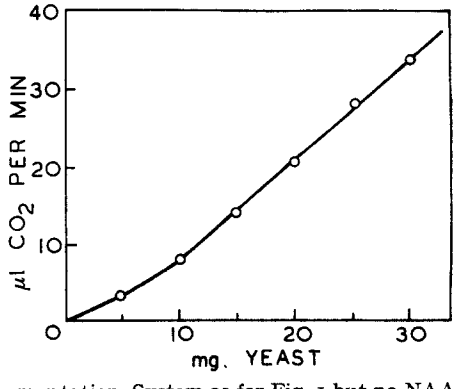
A. 0.005 *M* pyruvate + 0.03 *M* glucose.
B. 0.01 *M* pyruvate + 0.03 *M* glucose.
C. 0.005 *M* pyruvate + no glucose.
D. no pyruvate, + 0.03 *M* glucose.

Omission of ATP re-introduced a lag period whilst NAA (0.01 *M*) had no effect indicating the absence of significant DPNase activity in the yeast powder preparation. A very active fermentation proceeds indeed, without addition of DPN to the system, and addition of DPN has but little effect. It thus appears that the acetone-dried yeast preparation contains sufficient DPN to saturate the fermentation system.

For optimal conditions, the fermentation system contains 0.02 *M* K-phosphate buffer pH 6.0; 0.005 *M* $MgCl_2$; 0.001 *M* ATP; 0.03 *M* glucose; 0.005 *M* pyruvate; yeast suspension; water to total final volume of 3.0 ml. After gassing and temperature equilibration the glucose and pyruvate are tipped from the side-arm at zero time.

Increasing amounts of yeast, from 5 to 30 mg per vessel, each gave linear rates of $CO_2$ output for at least the first 30 minutes; at the higher levels of yeast the rate




Figs. 2 and 3. Effect of yeast concentration on rate of fermentation. System as for Fig. 1 but no NAA present and using 0.005 *M* pyruvate. A to F contained respectively 5, 10, 15, 20, 25 and 30 mg yeast.

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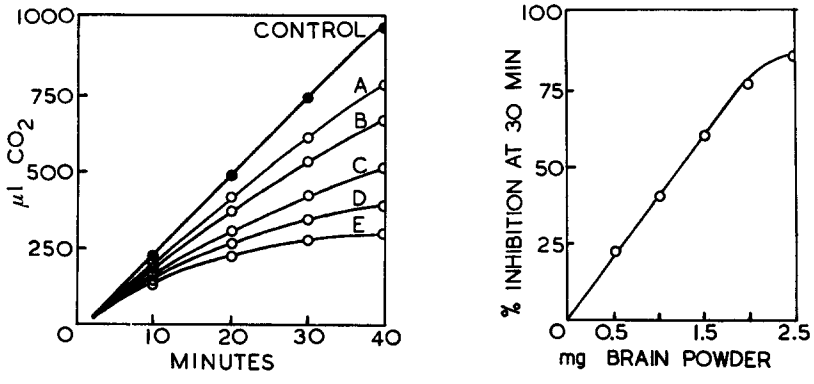
tends to decrease (Fig. 2). The initial slopes plotted against the weight of yeast gave the curve shown in Fig. 3—the relationship being linear above 10 mg per vessel. The final pH at the 30 mg level was 5.7 to 5.8; with less yeast the pH remained nearer the initial value of 6.0. In the experiments described below, 30 mg yeast was used with the optimal system.

*Effect of acetone-dried beef brain on fermentation at pH 6.0*

As was previously shown by HARPUR *et al.*[7], addition of an extract of acetone dried beef brain was found to inhibit the yeast fermentation. In order to study the phenomenon on a more quantitative basis the brain (and later, other tissues or extracts) was added last to the otherwise complete system and the time noted. Thirty minutes later, the fermentation was started by tipping the glucose and pyruvate from the side-arm and the gas output followed for the next 50 to 60 minutes. As shown by the results given in Fig. 4, addition of the brain suspension resulted in a progressive inhibition. The slopes of the curves at 30 minutes relative to the simultaneous control slope gives




Figs. 4 and 5. Effect of acetone-dried beef brain suspension on yeast fermentation. Control system as for Figs. 2 and 3 using 30 mg yeast; brain suspension added to main compartment 30 minutes before tipping the glucose and pyruvate. A to E contained respectively 0.5, 1.0, 1.5, 2.0 and 2.5 mg brain.

the percentage inhibition, and as shown in Fig. 5 when this is plotted against the weight of brain, a linear relationship obtains up to about 70% inhibition (1.8 mg brain powder). As the percentage inhibition increases, the final pH remains nearer the initial value; thus in the experiment of Fig. 5, for example, whilst the final pH of the control was 5.65, at 25% inhibition it was 5.70, at 41%, 5.80; 61%, 5.95; 75%, 6.00 and 84% 6.00. This suggests that under the present experimental conditions, only a minor fraction of the inhibition, if any, is due to diversion of the pyruvate to lactate. The observed pH changes are probably related for the most part to the volume of $CO_2$ liberated.

The inhibition was shown in two ways to be due almost entirely to the DPNase activity of the brain preparation. MANN AND QUASTEL[14] and many subsequent workers have shown that NAA in sufficiently high concentration will inhibit DPNase activity. When the brain was added to the system already containing 0.01 *M* NAA the inhibition of fermentation was almost completely antagonised. It should be noted, however, that when large amounts of brain are used the antagonism is less complete (see results given in Fig. 7, 40 mg fresh brain); indeed, with excessive amounts of brain the presence of nicotinamide is ineffective. As the inhibition of DPNase produced by NAA is not 100%,

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this is an expected result; with high concentrations of DPNase, although the percentage inhibition will remain the same for a given concentration of nicotinamide, the absolute residual activity of the enzyme is high enough to maintain a sub-optimal DPN concentration. The effect of a brain preparation on yeast fermentation and its antagonism by NAA will thus depend upon (i) the amount of DPNase present, and (ii) the time of incubation prior to tipping in the substrate.

Furthermore, the inhibiting effect of the brain can be reversed by tipping DPN at zero time together with the glucose and pyruvate as is shown by the results grown in Fig. 6. Even when excessive amounts of brain are used so that the presence of NAA affords no protection, addition of DPN at the end of the experiment results in a re-establishment of the fermentation.

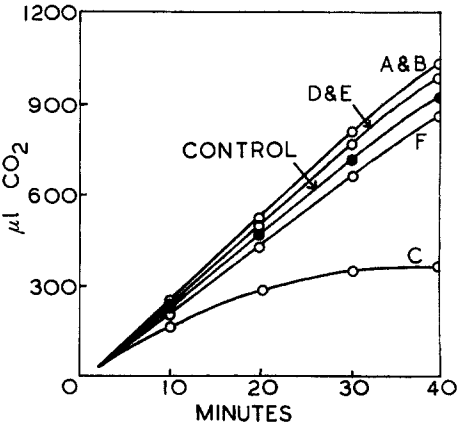


Fig. 6. Effects of DPN and NAA (0.01 *M*) on the inhibition of fermentation by acetone-dried beef brain suspension. Control system as for Figs. 2 and 3 using 30 mg yeast.
A. 1 mg DPN in side-arm.
B. 1 mg DPN + NAA in side-arm.
C. 2 mg brain in main compartment 30 min before tipping glucose and pyruvate.
D. as C but 1 mg DPN also in side-arm.
E. as C but 1 mg DPN + NAA also in side-arm.
F. as C but NAA in main compartment.

*Experiments with fresh tissue homogenates. The $I_{50}$ value.*

Readily reproducible results were obtained with the acetone-dried brain powder, and subsequent experiments using fresh guinea pig and rat brain homogenates yielded similar results (Fig. 7). The system, containing 40 mg fresh weight brain shows the incomplete antagonism by NAA referred to above.

When concentrations of tissue are used such that the inhibition of fermentation is almost completely antagonised by NAA, then the slopes of the curves after a given experimental ("post-tipping") period are a measure of the residual DPN in the system at that time. Thus, the slopes after a 30 minute experimental period were determined for a series of vessels containing increasing amounts of tissue and the percentage inhibition relative to a simultaneous control without added tissue was plotted against the dry weight of tissue used. The resulting curves were linear up to about 80% inhibition and typical results using beef brain (homogenates of grey and white matter separately) are shown in Fig. 8.

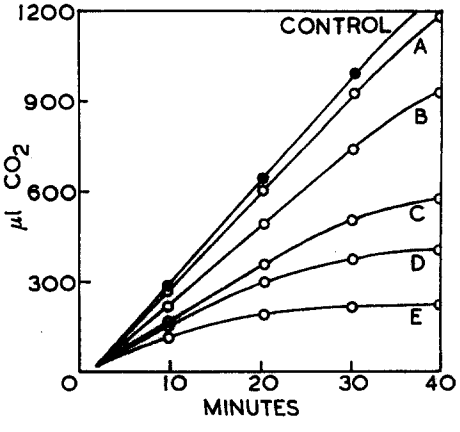


Fig. 7. Effect of guinea pig brain homogenate on yeast fermentation. Control system as for Figs. 2 and 3 using 30 mg yeast; brain (in terms of fresh weight) added to main compartment 30 min before tipping. Final concentration NAA when present, 0.01 *M*.

A. 10 mg brain + NAA.
B. 20 mg brain + NAA.
C. 40 mg brain + NAA.
D. 10 mg brain.
E. 20 mg brain.

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Such curves permit the determination of $I_{50}$ value, *i.e.* that amount of tissue in terms of mg dry weight which in a 30 minute experimental period reduces the DPN concentration to such a level that the rate of fermentation is reduced by 50%. The $I_{50}$ value for a tissue is thus inversely related to its DPNase content, and comparison of the values from one tissue to another gives a quantitative measure of their relative DPNase contents. In all such experiments a vessel was included which contained NAA; unless the inhibition by the tissue was antagonised by the NAA the result was considered as being due to some cause other than DPNase (nucleosidase) activity. It is interesting to note that the ratio of the $I_{50}$'s for grey and white matter of beef brain is 1.5:1 and is thus of the same order as the ratio found by McILWAIN AND RODNIGHT[19] for guinea-pig brain (approx. 2:1). A similar experiment with guinea pig brain gave $I_{50}$ values of 1.08 for grey matter and 0.54 for white—a ratio of 2:1. Furthermore, the $I_{50}$ for acetone-dried brain was 1.25 and as this is of the same order as the mean of the values for grey and white matter obtained above with fresh tissue, it suggests that little loss in activity occurs in the preparation of the acetone powder.

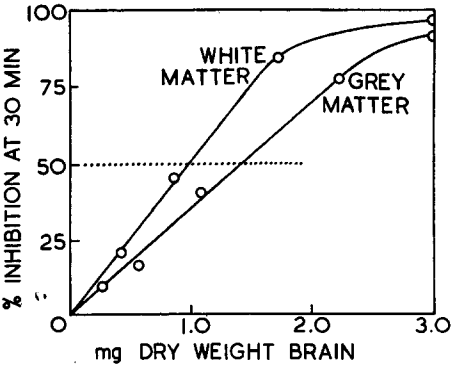



Fig. 8. $I_{50}$ values of grey and white matter of beef brain. Procedure as for Figs. 4 and 5.

TABLE I

$I_{50}$ VALUES FOR NORMAL MAMMALIAN TISSUES

| *Tissue* | $I_{50}$ *(mg dry weight)* | *Tissue* | $I_{50}$ *(mg dry weight)* |
|---|---|---|---|
| Guinea pig brain | | Guinea pig spleen | < 1.10; 0.20 |
| whole brain | < 2; < 2; 1.24 | | |
| white matter | 0.54 | Guinea pig lung | < 0.93; 0.27 |
| grey matter | 1.08 | | |
| Beef brain | | Guinea pig heart | 2.50 |
| white matter | 0.95 | | |
| grey matter | 1.42 | Guinea pig kidney | > 3.0; 5.20 |
| acetone dried | 1.25 | | |
| | | Guinea pig whole blood | > 18 |
| Rat brain | 1.45 | | |
| | | Guinea pig erythrocytes | > 137 |
| Human brain | *ca* 8.0 | | |
| cerebellum | < 8.0 | Rabbit testis | *ca* 4[a] |
| Guinea pig liver | 1.40; 1.90 | Pigeon erythrocytes | > 100 |
| Rat liver | 1.80 | | |
| Pigeon liver | > 7[c] | | |
| Beef retina | > 3.5[b] | | |

The inhibition produced by guinea pig muscle was insensitive to NAA.
[a]. Testis induced a lag period; the subsequent inhibition was NAA-sensitive.
[b]. Up to 3.5 mg dry weight retina had no effect; NAA-sensitive inhibition at higher levels.
[c]. Up to 7.0 mg dry weight pigeon liver had no effect; NAA-sensitive inhibition at higher levels.

*References p. 267.*

A series of experiments was carried out to determine the relative DPNase activities of various normal animal tissues. The results are shown in Table I. Of the tissues examined, guinea pig spleen and lung appear to have the highest activities whilst whole blood and erythrocytes (nucleated and non-nucleated) show very little.

Two preliminary results were obtained with normal human brain tissue excised during brain tumour operations. These gave $I_{50}$ values which indicate that human brain probably contains significantly less DPNase per unit dry weight than guinea-pig, rat or beef brain.

A NAA insensitive inhibition produced by retina homogenates was accompanied by a marked decrease in the final pH, suggesting that a high lactic dehydrogenase activity might be diverting the pyruvate to lactate. Muscle also produced a NAA-insensitive inhibition, whilst rabbit testis induced a lag period but the inhibition measured during the final steady rate of fermentation was antagonised by NAA. These observations were not further investigated.

*Experiments with tumour tissue homogenates*

Ten human brain tumours, four other tumours of human origin, transplanted mouse tumours (sarcoma 37) at various stages of growth, and the livers of rats fed a diet containing 0.06% butter yellow were examined for DPNase activity. All the inhibitions were nicotinamide-sensitive and the results are shown in Table II.

TABLE II

$I_{50}$ VALUES FOR TUMOUR TISSUES

| *Tumours* | $I_{50}$ *(mg dry weight)* |
|---|---|
| A. *Human brain tumours* | |
| 1. Unclassified cerebellar glioma (fibrous astrocytoma with malignant features) | > 24 |
| 2. Cerebellar fibrous astrocytoma | > 24 |
| 3. Perineurial fibroblastoma of 8th nerve | > 40 |
| 4. Glioblastoma multiforme of temporal lobe | 4.8 |
| 5. Unclassified glioma of temporal region (astrocytoma with malignant features) | 1.3 |
| 6. Glioblastoma multiforme of temporal lobe | 0.6 |
| 7. Malignant cerebellar tumour, type undiagnosed | > 13.2 |
| 8. Parietal fibrous astrocytoma | 0.48 |
| Adjacent invaded tissue | 1.05 |
| 9. Astrocytoma | 30.0 |
| 10. Metastatic lung tumour | 3.4 |
| B. *Other tumours of human origin* | |
| Cystic papillary adenoma of ovary | > 16.3 |
| "Skin" tumour | 0.8 |
| Adenocarcinoma of colon | 2.9 |
| Breast tumour | 24.0 |
| C. *Mouse sarcoma* 37 | |
| 8 days after transplantation; no necrosis | 1.5 |
| 16 days after transplantation; central core of necrotic tissue removed | 2.05 |
| 17 days after transplantation; central core of necrotic tissue removed | 1.70 |
| 28 days after transplantation; much necrotic tissue included | 2.4 |
| D. *Rat liver after feeding butter yellow* | |
| Liver from rat fed butter yellow (precancerous, no gross visual abnormalities after 100 days on diet) | 1.50 |
| Liver from rat fed butter yellow (mottled appearance and many nodules after 130 days on diet) | 1.75 |

*References p. 267.*

Whilst five of the brain tumours gave DPNase levels which lie within the range found for most normal tissues (Table I), the remainder showed surprisingly low activities. Of the four other tumours of human origin, two gave "normal" values whilst two gave very low ones. It is important to note that a proper interpretation of these values involves a knowledge of the pathological changes that may be taking place in the tumours themselves. The results obtained with the mouse sarcoma 37 showed little change over the period 8 to 28 days after transplantation. Finally, the livers from the rats fed butter yellow showed no significant difference compared with control rat livers either before the appearance of gross pathological signs (100 days on butter yellow) or when the liver had become grossly neoplastic (130 days on butter yellow + 15 days normal diet).

*Estimation of anti-DPNase activities of various substances*

Acetone-dried beef brain was used as the source of DPNase. Experiments with the whole aqueous suspension and with the supernatant obtained after centrifugation showed that about 80% of the activity remained associated with the residue. Incubation of the yeast system with increasing amounts of the brain suspension showed that the degree of inhibition of fermentation (30 min after tipping the substrate) was directly proportional to the amount of brain up to 75–80% inhibition (Fig. 4). This linear relationship between DPNase activity and fermentation rate thus provided a convenient system for the assay of DPNase inhibition by various substances. Two mg brain per vessel (giving 80% inhibition) was adopted in the standard system.

By this procedure, nicotinamide was shown to produce 50% inhibition of DPNase at $1.75 \cdot 10^{-3}$ *M*. This is in reasonable agreement with McILWAIN's value[17] of *ca* $1 \cdot 10^{-3}$ *M* obtained with a semi-purified cell-free enzyme system. With phenosafranine, however, no inhibition was observed at $7.4 \cdot 10^{-4}$ *M*, compared with the 62% inhibition at $6.7 \cdot 10^{-5}$ *M* observed by McILWAIN; higher concentrations of the dye inhibited the control yeast fermentation and made assays of the anti-DPNase activity impracticable. This difference in the inhibitory activity of phenosafranine is probably due to the high degree of adsorption which probably takes place in the yeast system. McILWAIN AND GRINYER[18] have shown that brain slices strongly adsorb the dye whilst, with nicotinamide, the concentration in the medium is relatively little affected.

GOVIER, YANZ AND GRELIS[4], GOVIER AND JETTER[3] and SPAULDING AND GRAHAM[24] claimed that α-tocopheryl phosphate inhibits DPNase activity. LE PAGE[13], however, observed that whilst 0.04 *M* nicotinamide markedly inhibited DPN breakdown in tumour homogenates, $6.7 \cdot 10^{-4}$ *M* α-tocopheryl phosphate afforded no such protection. When tested in the present system, up to $5 \cdot 10^{-3}$ *M* α-tocopheryl phosphate had no significant effect on the DPNase activity of either the acetone-dried beef brain preparation or a guinea pig liver homogenate, whilst 0.01 *M* nicotinamide gave 90–100% inhibition.

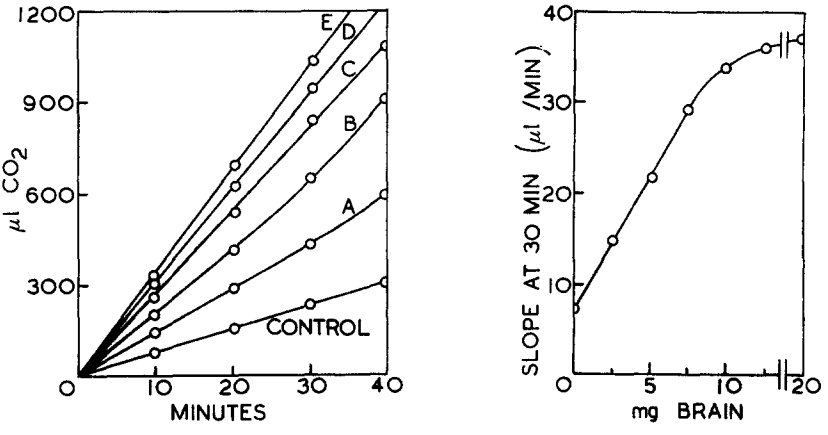
The following were amongst the substances tested for anti-DPNase activity, all giving negative results at the concentrations used: $2 \cdot 10^{-2}$ *M* benzamide; $2 \cdot 10^{-2}$ *M* pyridoxal; $2 \cdot 10^{-3}$ *M* muscle adenylic acid; $2 \cdot 10^{-3}$ *M* yeast adenylic acid; $1.6 \cdot 10^{-3}$ *M* prostigmine; $1 \cdot 10^{-3}$ *M* azaguanine; $5.8 \cdot 10^{-3}$ *M* atabrine; $3 \cdot 10^{-3}$ *M* methionine sulphoximine; 3 mg thyroxine; $2 \cdot 10^{-3}$ *M* sulphanilamide; 2 mg sulphapyridine; 2 mg stilbestrol; $10^{-3}$ *M* 2-acetylaminofluorene*; $10^{-3}$ *M* dimethylaminoazobenzene[3]; 1.65 *M* methanol.

* In 0.1 ml methanol.

*References p. 267.*

*Experiments at pH 7.4*

At pH 7.4 in 0.04 *M* $NaHCO_3$/7% $CO_2$ buffer, 30 mg yeast powder gave an insignificant gas output in the presence of 0.03 *M* glucose, 0.005 *M* pyruvate, 0.005 *M* $MgCl_2$ and 0.002 *M* phosphate, as the yeast carboxylase is inactive at this pH. Addition




Figs. 9 and 10. Effect of an extract of acetone-dried beef brain on the yeast system at pH 7.4 in the presence of 0.01 *M* NAA. Control system contains 30 mg yeast, 0.001 *M* ATP, 0.005 *M* $MgCl_2$, 0.04 *M* $KHCO_3$, 0.002 *M* K-phosphate pH 7.4 and 0.01 *M* NAA in the main compartment, and glucose (to give 0.03 *M* final), pyruvate (0.005 *M* final) and HDP (0.004 *M* final) in the side-arm. Total volume, 3.0 ml; temperature, 28° C; gas phase 93% $N_2$/7% $CO_2$. A to E contained respectively an extract from 2.5, 5.0, 7.5, 10.0, 12.5 and 20.0 mg brain powder.

of an extract of acetone dried brain together with 0.01 *M* NAA resulted in a rapid evolution of $CO_2$ after a 10-minute lag period. The presence of 0.04 *M* HDP completely abolished this lag period but simultaneously introduced a yeast "blank". Higher concentrations of brain increased this gas output up to a maximal level as shown in Fig. 9 and 10. Addition of the brain to the system in the absence of NAA, however, first increased the gas output and then, as the brain concentration was raised, inhibited it, as shown in Fig. 11. Heating either the brain or yeast preparations at 100° for 10 minutes resulted in complete loss of activity.

These results suggest that the effect of addition of brain extract to the yeast system at pH 7.4 is the net result of two antagonistic mechanisms;

1. a stimulatory effect due to the brain DPN-linked lactic dehydrogenase, affecting the removal of pyruvate and a re-oxidation of the $DPNH_2$.

2. an inhibitory effect due to the brain DPNase, destroying the DPN and inhibiting the lactate production and hence the $CO_2$ output.

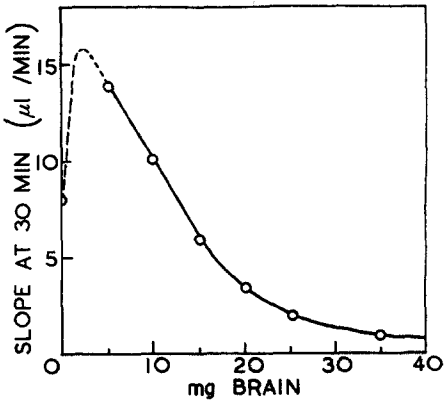



Fig. 11. Effect of an extract of acetone-dried beef brain on the yeast system at pH 7.4 in the absence of NAA. Details as for Figs. 9 and 10 but no NAA added. Brain extract added 30 minutes before tipping.

*References p. 267.*

Thus, at low concentrations of brain in the absence of NAA, mechanism (1) is predominant whilst, at higher concentrations mechanism (2) becomes increasingly effective until the $CO_2$ output is reduced almost to zero. Similar results are obtainable with fresh brain homogenates and the method could be developed as a possible means of estimation relative lactic dehydrogenase and DPNase activities, in a similar manner to the method adopted for DPNase at pH 6.0.

## DISCUSSION

The inhibition of yeast fermentation by animal tissues described by HARPUR *et al.* has been subjected to a more detailed and more quantitative examination. Under their experimental conditions, these authors observed a strong inhibition by brain extract which was only partially antagonised by nicotinamide and which was associated with an enhanced lactic acid formation. The situation is rather different, however, when the yeast is fermenting under optimum conditions at pH 6.0 and when the duration of the incubation of the yeast and brain is carefully controlled. In these circumstances, the tissue lactic dehydrogenase appears to play only a minor role and the inhibition is almost completely antagonised by nicotinamide. The lactic dehydrogenase effect is best studied at pH 7.4 in a bicarbonate medium as under these conditions the yeast carboxylase is completely inactive. Addition of low concentrations of brain to such a system results in a large gas output, but as the brain concentration is increased this gas evolution becomes entirely dependent on the presence of nicotinamide owing to the intervention of the brain DPNase.

With the majority of the tissues examined the inhibition of the yeast fermentation system at pH 6.0 is almost completely antagonised by nicotinamide, indicating that the DPN is being split at the nicotinamide-ribose bond (nucleosidase activity). As nicotinamide is apparently a specific inhibitor of this enzyme, it appears that the nucleosidase is the enzyme predominantly responsible for DPN cleavage in most of the tissues examined, whilst in guinea pig muscle, beef retina and pigeon liver the pyrophosphatase or some other enzyme may be partly responsible (assuming that the inhibition with these tissues is due to DPN destruction, a point not investigated here). It is also interesting that of the many substances tested for anti-DPNase activity in the present system, NAA is the only one which has any significant action.

Variations in DPNase activity were observed amongst the various normal animal tissues examined, although apart from whole blood and erythrocytes (guinea pig and pigeon) and pigeon liver, the $I_{50}$ values were between 0.2 and 5.2; the value for pigeon liver of 10.0 indicates a surprisingly low activity. The results with the tumours are of interest in view of GREENSTEIN's thesis[5] that tumours possess a more uniform and less diverse chemical pattern than normal tissues and that this pattern seems to be independent of aetiology or histogenesis. Our results, although rather limited in number, show a much wider scatter of DPNase levels amongst the various tumours than was found amongst the normal tissues. Such findings make further investigation of interest, especially if the DPNase activity of a tumour can also be correlated with the rate of proliferation, malignancy or some other property of neoplastic tissue. A change in the DPNase activity of a tissue might be expected to upset the balance of energy-yielding reactions and such a correlation would provide an invaluable lead in the study of tumour metabolism.

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SUMMARY

1. Optimal conditions have been defined for the fermentation of glucose by an acetone powder of yeast extract at pH 6.0. The initial lag period before the onset of fermentation is eliminated by addition of a small amount of pyruvate.

2. The inhibitory effect of an extract of an acetone-dried powder of beef brain on the fermentation at pH 6.0 was studied and shown to be due almost entirely to DPNase (nucleosidase) activity. This phenomenon has been developed on a quantitative basis and used as a method of estimation of the relative DPNase activities in normal and pathological tissues.

3. Guinea pig spleen and lung showed the highest DPNase activity per unit dry weight amongst the normal tissues whilst guinea pig kidney and pigeon liver showed rather low activities. Although variations in the levels were observed amongst both normal and tumour tissues, those obtained for the tumours appear to be spread over a much wider range than those for the normal tissues.

4. The yeast/brain system at pH 6.0 was further adapted for the assay of anti-DPNase activity. α-Tocopheryl phosphate, benzamide and phenosafranine and numerous other substances were inactive in protecting the DPN; nicotinamide was the only compound tested which showed any significant anti-DPNase activity.

5. The effect of addition of a brain extract to the yeast system at pH 7.4 is the net result of a stimulatory effect due to the brain DPN-linked lactic dehydrogenase and an inhibitory effect due to the brain DPNase.

RÉSUMÉ

1. Nous avons défini les conditions optimales de la fermentation du glucose par un extrait de levure (poudre séchée à l'acétone) à pH 6.0. La période initiale précédant le début de la fermentation peut être éliminée par addition d'une petite quantité de pyruvate.

2. Nous avons étudié l'effet inhibiteur exercé sur la fermentation à pH 6.0 par un extrait d'une poudre (séchée à l'actone) de cerveau de boeuf; cet effet est dû presque exclusivement à l'activité de DPNase (nucléosidase). Ce phénomène a été étudié quantitativement; il est à la base d'une nouvelle méthode de détermination des activités de DPNase relatives de tissus normaux et pathologiques.

3. Parmi les tissus normaux la rate et le poumon de cobaye montre la plus forte activité de DPNase par unité de poids sec, tandisque le rein de cobaye et le foie de pigeon ont des activités plutôt faibles. Quoique des variations de niveau aient été observées aussi bien parmi les tissus normaux que parmi les tissus cancéreux, les équarts semblent beaucoup plus considérables parmi les derniers.

4. Le système levure/cerveau à pH 6.0 a été, de plus, employé pour un test de l'activité anti-DPNase. Le phosphate d'α-tocophéryl, la benzamide, la phénosafranine et de nombreuses autres substances ne protègeaient pas le DPN; la nicotinamide était la seule substance étudiée montrant une activité anti-DPNase significative.

5. L'effet de l'addition d'un extrait de cerveau au système de levure à pH 7.4 est le résultat net d'un effet stimulant dû à la déhydrogénase lactique liée au DPN du cerveau et d'un effet inhibiteur dû à la DPNase du cerveau.

ZUSAMMENFASSUNG

1. Die besten Bedingungen für die Glucosegärung unter dem Einflusse eines mit Aceton getrockneten Hefeextrakt-Pulvers bei pH 6.0 wurden umschrieben. Die Verzögerung des Gärungsbeginnes kann durch Zugabe einer kleinen Menge Pyruvat aufgehoben werden.

2. Die Hemmwirkung eines Extraktes von (mit Aceton getrocknetem) Ochsenhirnpulver auf die Gärung bei pH 6.0 wurde untersucht; sie ist beinahe vollständig auf die DPNase (Nukleosidase)-

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Aktivität zurückzuführen. Diese Erscheinung wurde quantitativ studiert; sie bildet die Grundlage einer Bestimmungsmethode der relativen DPNase-Aktivitäten normaler und pathologischer Gewebe.

3. Meerschweinchenmilz und -lunge zeigten unter den untersuchten normalen Geweben die höchste DPNase-Aktivität pro Trockengewichtseinheit, während Meerschweinchenniere und Taubenleber eher niedrige Werte ergaben. Obgleich Variationen sowohl bei den normalen wie bei den Tumorgeweben beobachtet wurden, lagen für Tumoren die Werte doch viel weiter auseinander.

4. Das Hefe/Hirn-System bei pH 6.0 wurde auch für die Bestimmung der Anti-DPNase-Aktivität angepasst. α-Tocopherylphosphat, Benzamid, Phenosafranin und zahlreiche andere Substanzen schützten DPN nicht; Nicotinamid war die einzige der untersuchten Verbindungen, welche eine signifikante Anti-DPNase-Aktivität zeigte.

5. Die Wirkung der Zugabe von Hirnextrakt zu dem Hefesystem bei pH 7.4 ist das Ergebnis des stimulierenden Effektes der an Hirn-DPN gebundenen Milchsäure-Dehydrogenase und des Hemmeffektes der Hirn-DPNase.

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