PHASED INJECTION IN DIESEL ENGINES

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Received on February 10, 1960

Abstract

It is well known that one of the main factors affecting the performance of Diesel engines has been the short time available for proper mixing of fuel and air. It is considered that the phased injection of the total fuel charge will bring about better mixing of fuel and air and in consequence lower specific fuel consumption, combustion noise, smoke density, etc. This paper describes the experiments conducted on a high speed, single cylinder diesel engine running on different fuels utilizing "phased injection system".

Introduction

The aim of diesel engine designers has always been directed towards obtaining low specific fuel consumption under all operating conditions and smoke free performance of the engine. In recent years several investigators have in addition, engaged their attention on the aspect of reducing the combustion noise and successful operation of the engine on several grades of liquid fuels so as to make it insensitive to the type of fuel used.

The stumbling block to the above improvements in a diesel engine has been mainly due to (*a*) ignition delay and (*b*) poor air utilization. Earlier investigators have obtained a reduction in the delay period of the engine by using a higher Cetane number fuel[1] or adding certain chemical substances[2] to the diesel fuel or increasing the Compression ratio[3] of the engine. Several investigators[4—11] have studied the performance of diesel engines by admitting a portion of the fuel—this fuel either being the same type as the main charge or of a different type—into the intake manifold or by direct injection into the engine cylinder. In all the above cases the remainder of the fuel charge was injected in the normal way, *i.e*, very near the top dead centre in the compression stroke. Results obtained by all the investigators have shown a decrease in the delay period and an increase in the air utilization factor of the engine.

The use of higher Cetane number fuel or the addition of chemical substances to the diesel fuel or the increase of the Compression ratio, reduces the delay period only to a limited extent. The addition of supplementary fuel through the induction manifold though efficient in regard to air utilization and reduction of delay period, suffers from the disadvantage that provision has to be made for the use of extra equipment in the form of a Carburretor, Micro-fog or Vaporizer, etc Further, the supplementary fuel addition leading to bi-fuel system as adopted by some investigators presents fuel feed and storage problems for the two fuels.

Object of the Test

The object of the test was to evolve the particular type of "phased injection system" using only one type of fuel which would eliminate the bi-fuel system and also the use of an extra equipment. The "phased injection system" consists of injecting the total fuel in two parts, the first part being injected well in advance of the second part, while the second part is injected in the normal fashion. It was intended for experimental purposes to use an auxilary fuel injector pump to inject the first part of the fuel It is however expected that a suitable design of the fuel cam and the pump can eliminate the need for an extra fuel injection pump.

In such an arrangement, the first part of the total fuel, injected fairly in advance of the second part would mix well with the air in the cylinder before the temperature of air reaches the self-ignition temperature of the fuel. Hence the air-fuel ratio of the first part of the fuel would be below the limit of inflamability for ignition, but will undergo initial oxidation resulting in an increase of pressure and temperature which would be favourable for the complete combustion of the second part of the total fuel. It is expected that a decrease in the ignition delay and combustion noise would be possible due to higher pressures and temperatures resulting from the preflame reactions of the initial charge of the fuel Further, the better mixing of fuel and air, due to the larger duration of the mixing period for the supplementary fuel is expected to improve the air utilization of the engine and thus decrease the smoke density. The exact roles played by the first part of the fuel (which may also be termed as the supplementary fuel or pilot fuel) and the second part of the total fuel (which may be termed also as the main fuel) in respect of the variables such as the main fuel injection timing, proportion of pilot and main fuels, brake load, compression ratio, etc, were intended to be investigated.

Test Set-up

Plate I shows the details of the test set-up. The tests were conducted on a single cylinder Kirloskar AV 1, series II engine. Tables I and II give the detailed specifications of the engine and the fuel oil used respectively. The engine was mounted on a heavy test-bed and the engine shaft was connected to a swinging field electric dynamometer through a flexible coupling for power measurements

(*a*) *Modification to the fuel system*:—The main fuel to the engine was fed from a graduated burette to the main injection pump which was originally incorporated in the engine. The output from this pump was fed to the atomizer through a suitable non-return valve. The main fuel rate was obtained by noting the time taken to consume 25 cc. of the fuel.

TABLE I

Specifications of "Kirloskar" High Speed Diesel Engine
Type AV1, Series II

General Details	Four stroke, compression ignition, vertical, cold starting water cooled
Number of cylinders	One
Bore diameter	3.15" (80 mm)
Stroke	4.33" (110 mm.)
Swept volume	33.93 cu. in (553 cc.)
Compression ratio	16.5 : 1
Speed	1,500 rpm
Rated power	5 H. P.
Valve tappet clearance	0.007 in.
Inlet valve opens	$4\frac{1}{2}$ degrees b. t. d. c.
Inlet valve closes	$35\frac{1}{2}$ degrees after b. d. c.
Exhaust valve opens	$35\frac{1}{2}$ degrees before b. d. c.
Exhaust valve closes	$4\frac{1}{2}$ degrees after t. d. c.
Fuel injection pressure	2,500 lbs/sq. in.
Fuel injection timing (by spill)	24° before t. d. c.
Injection equipment	"Bryce" type A1AA70/5S 99K fuel pump "Bryce" No. AL 67 SD249 nozzle holder "Bryce" No UFHLS26C175P3 nozzle
Combustion chamber	Open combustion chamber
Fuel recommended	A high grade light distillate diesel fuel in accordance with B. S. S. 209/1947-Class 'A'
Lubricating oil recommended	Shell talpa S. A. E 30

TABLE II

Properties of High Speed Diesel Oil used in Tests

Specific gravity 60/60°F	0.840
Cetane number	55
Viscosity at 140°F Redwood No. 1 secs.	31
Carbon residue Conradson % weight	0.05
Sulphur % weight	0.3
Water % volume	Nil
Sediment % weight	Nil
Ash % weight	Nil
Calorific value-B T U /lb	
Higher	19,600
Lower	18,600

An additional fuel pump of the same type as the main fuel pump was driven by a camshaft connected directly to the camshaft drive of the engine through a flange coupling The delivery from this pump was connected to the same atomizer as that fed from the main fuel pump, through a suitable non-return valve The fuel for this additional fuel pump was fed from another calibrated burette The output from the additional or supplementary fuel pump was accurately adjusted by a micrometer fitted to the fuel pump rack of the pump. At any given setting of the supplementary fuel pump, the main fuel was automatically adjusted by the governor which was originally incorporated in the engine to maintain the speed of the engine at 1,500 rpm.

(*b*) *Cylinder pressure diagram* :—A condenser type of pick-up was installed in the cylinder head of the engine at a suitable point and the output from the pick-up was fed to a 'Mini-rack' double beam Cathode ray oscilloscope. The diagram obtained on the screen of the oscilloscope was photographed with the aid of a Universal Camera attached to the oscilloscope.

(*c*) *Injection timing* —The exact point of injection of the fuel into the combustion chamber was determined by a set of contact points mounted on the top of the nozzle and actuated by the rise of the pintle at the start of each injection The pulse generated by the opening of the contact points at the instant of injection was fed to the oscilloscope which gave a pip on the indicator diagram at the point of injection. This was utilised to measure the ignition delay which was the distance (in degrees) from the injection pip to the start of pressure rise.

The injection timing of the main fuel pump was varied by altering the thickness of the shims provided at the bottom of the fuel pump. The injection timing of the supplementary fuel pump was altered by varying the relative angular positions of the fuel pump camshaft with respect to the driving shaft.

(*d*) *Noise level*:—A magnetic microphone was clamped to the Cylinder head of the engine and the induced electromotive force was fed to the input circuit of the second beam of the oscilloscope.

Other standard instruments were used to measure the temparature of the exhaust gases, cooling water, etc. The exhaust smoke density was estimated by a C R. C. smoke meter.

Tests on the Engine with Supplementary Fuel Injection

During these tests the main fuel injection timing was kept at 9°* before the top dead centre as this was found to be the optimum timing while operating

*All the injection timings referred to in this paper are actual timings of injection as indicated by the contact points mounted on the top of the atomizer.

the engine on the main fuel alone. High speed diesel oil was used both for main and supplementary injections.

The initial experiments with the supplementary fuel injection during the suction stroke and upto 120° before the top dead centre in the compression stroke usually resulted in heavy crankcase dilution and deterioration in the performance of the engine. With the suplementary fuel injection at 90° before top dead centre in the compression stroke the unburnt diesel fuel vapours in the exhaust as observed in previous timings were absent but a whitish smoke was observed. Besides, with the supplementary fuel rate of about 80 % of the total fuel, knocking of the engine was audiable. Hence, it was felt that the addition of the supplementary fuel during the latter half of the compression stroke starting from 90° before top dead centre would be of great interest and it was decided to inject the supplementary fuel at closer intervals and also to find out the effect of supplementary fuel injection on brake loads ranging from part load to over load conditions. Hence, with the main fuel injection at 9° before top dead centre, the supplementary fuel was injected at angles of 90°, 70°, 48°, 36° before top dead centre and at every supplementary fuel injection timings as mentioned above, constant load tests were conducted at brake mean effective pressures of 33.35 lbs / sq in , 49.9 lb / sqs. in , 66.7 lbs / sq. in., and 93.3 lbs / sq. in., representing half load, three-fourths load, full load and 40 % over load respectively. An additional constant load test at 106 5 lbs/sq. in., bmep (60 % overload) was carried out with the supplementary fuel injection timing of 36° before top dead centre. At each brake load the supplementary fuel rate was varied over a wide range, the maximum being limited to the knocking conditions of the engine The effect of supplementary fuel injection on parameters like specific fuel consumption, smoke density, exhaust temperature, combustion pressure, ignition delay, engine noise level and the maximum rate of pressure rise was studied. The engine did not run steadily with the supplementary fuel injection retarded further to 30° before top dead centre and hence the tests were confined only to the supplementary fuel injection timings mentioned previously.

Tests results:—Figures I and II show the indicator diagrams obtained with supplementary fuel injection at 90° before top dead centre and at full load and half load respectively. Figures III and IV show the indicator diagrams obtained with supplementary fuel injection at 36° before top dead centre and at full load and half load respectively. Figures V to IX give the supplementary fuel injection *vs.* specific fuel consumption, exhaust temperature, smoke density, delay period and maximum rate of pressure rise repectively. Plate II shows the Indicator diagram and noise level diagrams with and without supplementary fuel injection The results are summarized below:

1. At a given supplementary fuel injection timing the amount of supplementary fuel that could be injected into the engine without knocking decreased with the increase of brake load.

2. Under part load conditions the specific fuel consumption and the exhaust temperature gradually increased with the supplementary fuel rate for all supplementary fuel injection timings. However, the increase in the specific fuel consumption was found to be least with a supplementary fuel injection timing of 36° before top dead centre.

3. At full load and overload conditions, injection of supplementary fuel in small quantities reduced the exhaust temperature and specific fuel consumption. The maximum beneficial effects were obtained at a supplementary fuel injection timing of 36° before top dead centre.

4 In general the delay period gradually decreased with the injection of supplementary fuel. The reduction of the delay period was faster at higher brake loads and with the supplementary fuel injection timing of 36° before top dead centre.

5. At a given brake load the peak pressure was found to vary only slightly with supplementary fuel injection. The peak pressure was found to occur a few degrees earlier with the injection of supplementary fuel. The rate of pressure rise was found to decrease slightly with the supplementary fuel injection.

6. The injection of supplementary fuel reduced the smoke density upto a minimum and thereafter increased slightly with further increase in the supplementary fuel rate.

7. The noise level of the engine reduced considerably with the injection of supplementary fuel.

8. In general, a supplementary fuel injection of about 15% to 20% of the total fuel was found to give satisfactory performance of the engine under all conditions of brake load.

Effect of Variation of Main Injection Timing

In order to determine the influence of the main fuel injection timing on the performance of the engine, constant load tests were conducted at brake mean effective pressures of 66.7 lbs/sq. in. (full load) and 93.3 lbs/sq. in. (40% overload) with the main fuel injection timing at 13° before top dead centre and 5° before top dead centre, keeping the supplementary fuel injection at 36° before top dead centre.

Test results:—Figure X shows the specific fuel consumption *vs.* supplementary fuel injection with the variation of main fuel injection timings for full load and overload conditions. The results are summarized below:

1. Under the same conditions of brake load the amount of supplementary fuel that could be injected into the engine without knocking was higher as the main fuel injection timing was retarded towards the top dead centre of the engine.

2. The performance of the engine in respect of the specific fuel consumption, etc., with the main fuel injection timing of 13° before top dead centre was almost the same as with the main injection timing of 9° before top dead centre. But the performance of the engine with a main fuel injection timing

of 5° before top dead centre deteriorated while working on the main fuel alone and the improvement obtained with the supplementary fuel injection did not compare well with the performance of the engine working on main fuel alone, with the fuel injection at 9° before top dead centre.

EFFECT OF VARIATION OF COMPRESSION RATIO

Since it could be expected that both compression pressure and temperature would affect the reactions of the supplementary fuel spray, constant load tests were conducted at full load and overload conditions with compression ratios of 17.5 and 14.7 while the normal compression ratio of the engine as in the previous tests was 16.5. The compression ratio of the engine was varied by increasing or decreasing the thickness of the cylinder head gaskets. The compression pressure was found to be as follows :

Calculated Compression Ratio	Measured Compression Pressure psi
14 7	385
16.5	430
17.5	460

During the tests the main fuel was injected at 13° before top dead centre and the supplementary fuel at 36° before top dead centre.

Test results:—Figure XI shows the specific fuel consumption vs. compression ratio for full load and overload conditions. The results are summarized below :

1. Under the same conditions of brake load, the maximum percentage of supplementary fuel that could be injected into the engine without knocking was higher as the compression ratio was reduced.
2. The beneficial effect in regard to the specific fuel consumption was slightly higher at higher compressio ratios.
3. The performance of the engine deteriorated on main fuel alone with a compression ratio of 14.7 and the beneficial effects obtained with the supplementary fuel injection did not compare well with the performance of the engine operating with a compression ratio of 16.5.

EXPERIMENTS WITH 'KEROSENE' AS THE FUEL

During these experiments the main fuel was injected at 13° before top dead centre and the supplementary fuel at 36° before top dead centre. The compression ratio of the engine was kept at 16.5. Table III shows the properties of kerosene used during the tests. Fig. XII and Plate IV show the results obtained by using kerosene for the main and supplementary fuel systems. The results indicate that the engine could be operated on optimum conditions with about 20% to 25% of the total fuel injected in the secondary system.

TABLE III

Properties of Kerosene used in Tests

Specific gravity 60/60°F	0 793
Cetane number	49
Viscosity at 140°F Redwood No. 1 secs.	27
Carbon residue % by weight	0 042
Water % Volume	Nil
Sediment % Weight	Nil
Calorific Value -B T. U. (Lower)	18,720

EXPERIMENTS WITH 'LIGHT DIESEL OIL' AS THE FUEL

During these experiments the main fuel was injected at 13° before top dead centre and the supplementary fuel at 36° before top dead centre. The compression ratio of the engine was kept at 16.5 Table IV shows the properties of the light diesel oil used during the test. Fig. XIII and Plate IV shows the results obtained by using light diesel oil for the main supplementary systems.

TABLE IV

Properties of Light Diesel Oil used in Tests

Specific gravity 60/60°F	0.897
Cetane number	47
Viscosity at 140°F Redwood No. 1 secs.	36
Carbon residue % Weight	0 61
Water % Volume	Nil
Sediment % Weight	Nil
Calorific Value B. T. U/lb (Lower)	18,000

It can be seen from the results that the specific fuel consumption improved at full load and overload conditions but increased under part load conditions. A reduction in the delay period, noise level and smoke density was also obtained with the addition of the supplementary fuel. The results indicate that about 20% to 25% of the total fuel injected in the supplementary system would yield best results.

NON-FIRING TEST

This test was conducted in order to obtain the conditions inside the engine cylinder when only the supplementary fuel was injected. The following test procedure was adopted during this test : The engine was motored at 1,500 rpm until it was sufficiently warmed up. The supplementary fuel was gradually admitted and the fuel rate was increased slowly until knocking (if any) was heard.

Readings of exhaust temperature and fuel rate were recorded. Plate V shows the super-imposed pressure diagrams without and with a supplementary fuel rate of 0 892 lbs/hr. injected at 36° before top dead centre using High speed diesel oil. Other test results are presented below:

Compression ratio	Supplmentary fuel injection timing Degrees before tdc.	Fuel	Maximum fuel rate lbs/hr.	Increase in Compression Pressure	Increase in Temperature Exhaust F
16 5	90	H S D.*	1 8†	Negligible	10
16 5	70	H S. D	1 496	28	60
16.5	48	H S. D.	1.1	70	110
16 5	36	H. S D.	0 892	98	165
14 7	36	H S D.	1.0	92	160
17.5	36	H. S. D	0 516	90	162
16 5	36	Kerosine	0 597	94	170
16 5	36	Light Diesel oil	906	91	175

* High speed diesel oil.

† No knocking was heard even with this fuel rate.

DISCUSSION

The increase in the compression pressure with the supplementary fuel injection as obtained by the non-firing tests may be attributed to the slow oxidation of the supplementary fuel which is an exothermic process. The intermediate products of combustion such as the aldehydes and peroxides will hasten the combustion process. These reactions result in shortening of the delay period of the main fuel and are also responsible for the earlier occurrence of the peak pressure. The reduction in the delay period is responsible for lower rate of pressure rise and consequent smooth running of the engine. It is said[12] that beyond a maximum rate of pressure rise of 50 psi per degree crank-angle the engine would tend to knock. However, it was interesting to note that while the engine was run on Kerosene (Plate III) the running of the engine was very smooth (as evidenced by the noise level diagram) even though the maximum rate of pressure rise was as high as 68.6 psi per degree crankangle. It is felt that the acceleration of pressure rise plays a greater part in regard to the smooth running of the engine than the rate of pressure rise. Further investigation may be necessary in this direction.

The supplementary fuel, injected earlier in the cycle, has ample time to disperse well throughout the cylinder and by the time the pressure and temperature are high enough in the compression stroke for ignition, it forms a very lean mixture for ignition and undergoes only partial oxidation even though more fuel than the quantity required for idling of the engine under normal conditions is

admitted. The better mixing of fuel and air inside the cylinder has been responsible in improving the combustion of the engine resulting in a lower specific fuel consumption, smoke density, etc Our findings regarding the increase of specific fuel consumption under part load conditions and subsequent improvement in specific fuel consumption under high loads agree with those of Lyn[4] who states; 'The incomplete combustion of the intake fuel (in our case the supplementary fuel) is the main cause of the higher fuel consumption at light load At high loads, the luminous combustion of the main fuel takes a longer time than at lower load and so more of the intake fuel may proceed to complete combustion. Moreover, the same loss of intake spray represents a smaller proportion of the total fuel'.

It is interesting to note that the maximum fuel rate as given by the non-firing tests was not the limiting supplementary fuel rate under actual working conditions. For example, while motoring tests were done with high speed diesel oil, the permissible maximum fuel rate without knocking was found to be 0.892 lbs/hr. Any further increase in the supplementary fuel rate would result in heavy knocking of the engine. But with the engine running under full load conditions, a supplementary fuel rate of 1.284 lbs/hr was possible without any knocking of the engine. This increase in the supplementary fuel rate under engine operating conditions may be attributed to the burning of the partially oxidized supplementary fuel resulting from the ignition of the main fuel, before the incidence of knocking.

Conclusions

From the foregoing experiments it can be seen that the 'phased injection' system affords an efficient means of reducing the smoke density and the delay period of a diesel engine. These beneficial effects are also accompanied by a reduction in the specific fuel consumption under full load and overload conditions. The reduction in the smoke density of the engine makes it possible to increase the smoke limited output also. It can be concluded that the 'phased injection system' with about 20% to 25% of the total fuel injected in the supplementary system makes it possible for a high speed diesel engine to work satisfactorily on fuels of low cetane number. Though a separate fuel pump has been used for supplementary fuel injection during the experimental work, it is expected that a suitable design of the fuel cam and the injector pump can eliminate the need for an additional injector pump.

Acknowledgement

The authors wish to express their thanks to Dr. H. A. Havemann for suggesting this problem and to Prof. M. R. K. Rao for helpful discussions.

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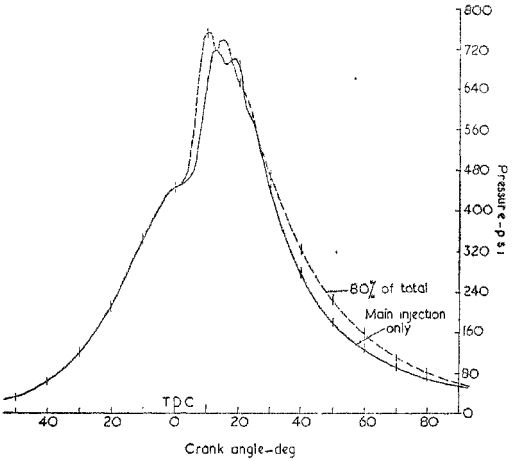


FIG. I

66.7 B. M. E. P (Full Load) Main - 9° B T. D. C. Pilot : 90° B. T D. C

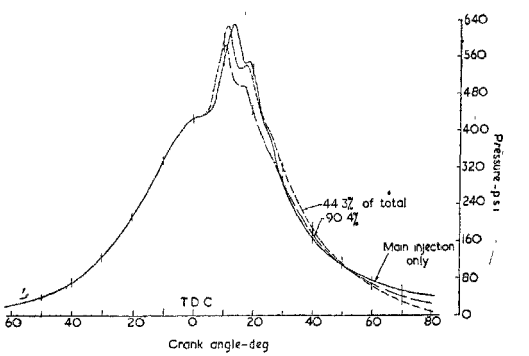


FIG II

33.35 B. M. E. P. Main . 9° B. T. D. C. Pilot : 90° B. T. D. C.

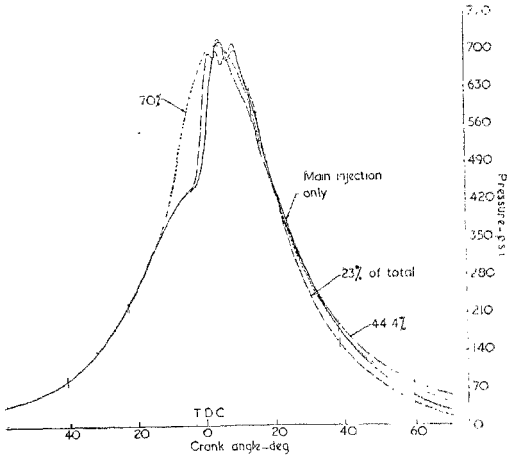


FIG III

66.7 B M. E. P. (Full Load) Main 9° B. T. D. C. Pilot: 36° B. T. D. C.

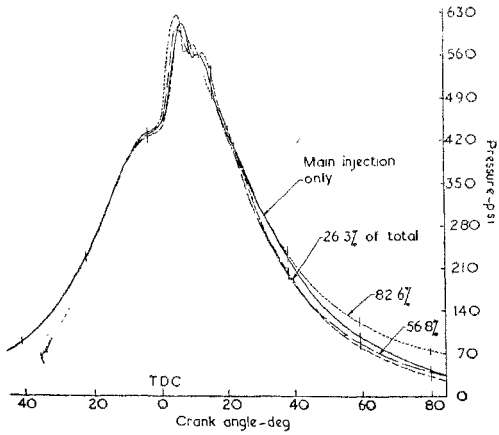


FIG. IV

33.35 B. M. E. P. Main 9° B. T. D. C. Pilot. 36° B T. D.

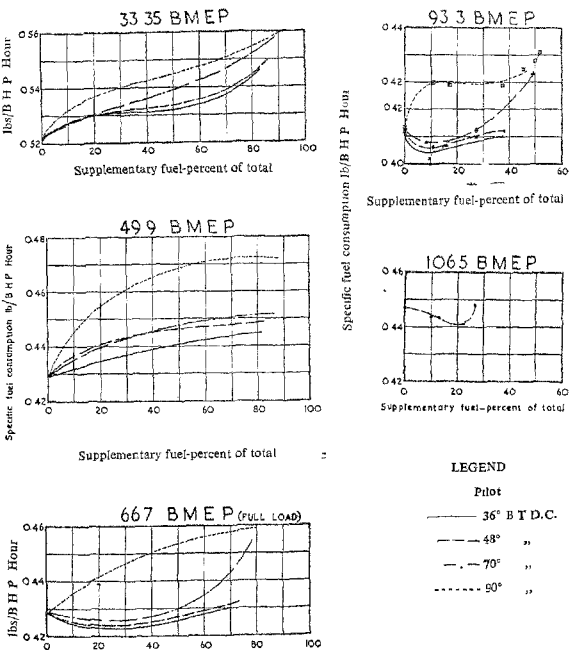


FIG. V

Main · 9° B.T D.C

Specific fuel consumption *vs* supplementary fuel at various supplementary or pilot fuel injection timings

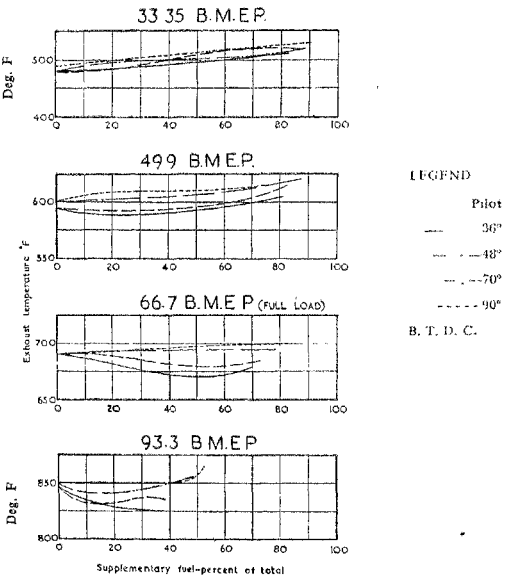


FIG. VI

Main: 9° B. T. D. C.

Exhaust temperature *vs.* supplementary fuel at various pilot fuel injection timings

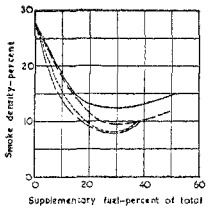


FIG VII

Main 9° B.T.D C. 93 3 B.M E P

Smoke density *vs.* supplementary fuel at various pilot fuel injection timings

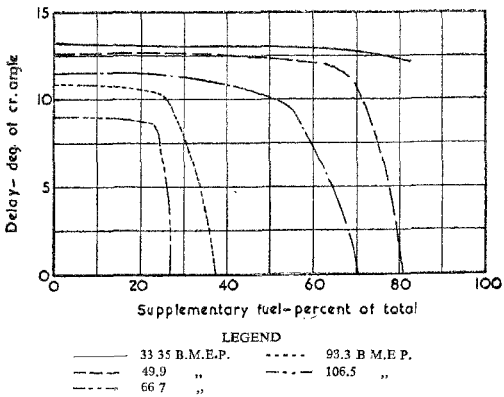
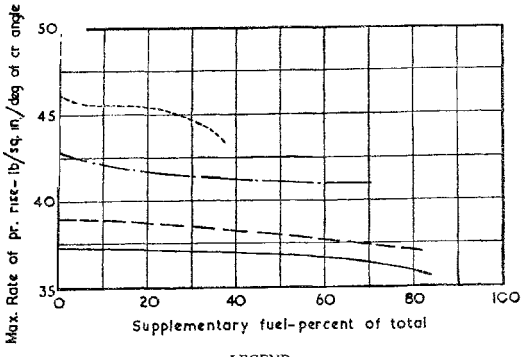


FIG. VIII

Main— 9° B.T.D C. Pilot—36° B.T.D C.

Delay period *vs* Supplementary fuel for various brake loads

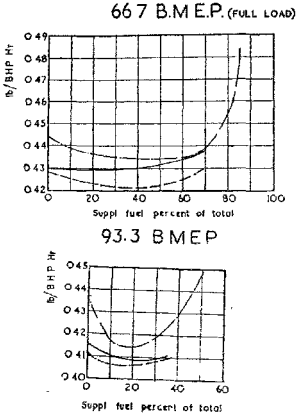


LEGEND

— — 33 35 B.M.E P — . — 66.7 B M.E P.
— — — 49.9 ,, ----- 93.3 ,,

FIG. IX

Main: 9° B T.D.C. Pilot: 36° B T.D.C.
Maximum rate of pressure rise *vs.* supplementary fuel at various brake loads

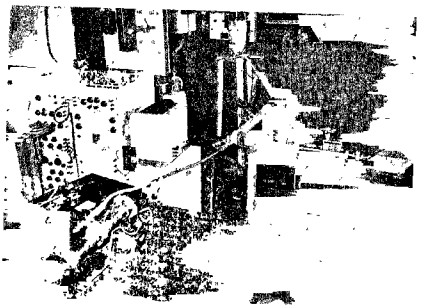


LEGEND

——— — Main 13° B T D C.
— — — 9° ,,
— . — 5° ,,

FIG. X

Pilot 36° B.T.D C. 66 7 B M E P. (Full Load)
Specific fuel consumption *vs* supplementary fuel at various main fuel injection timings



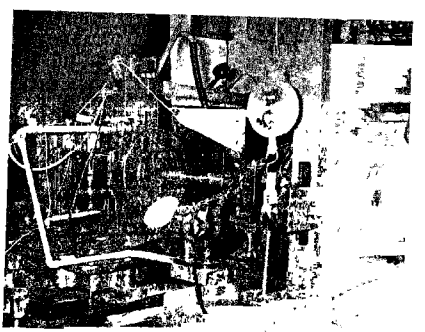
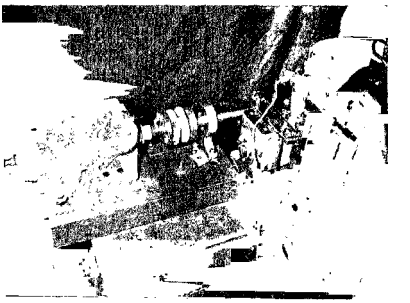


PLATE I *a* & *b*
General views of the test set-up

Close up view of the atomizer nozzle and cylinder head pick-up

Close up view of supplementary fuel system and noise level pick-up



Close up view of the time sweep unit

PLATE I (*contd.*) *c, d & e*

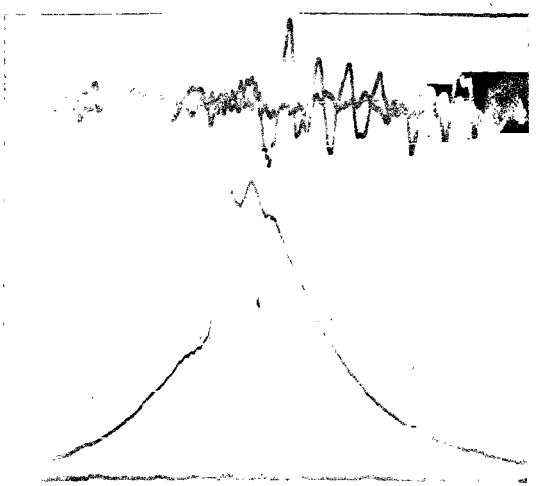


PLATE II *a*

Indicator diagram and noise level diagram of the engine running at 1500 R P M at a brake load of 93 3 lbs/sq in bmep without supplementary fuel injection. Main injection 9° before top dead centre

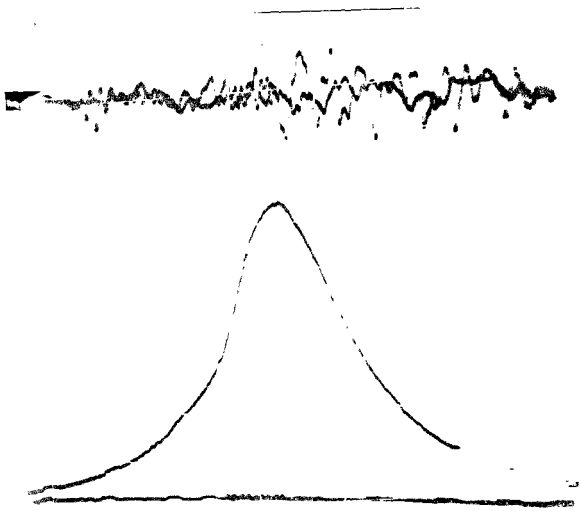


PLATE II *b* (*contd*)

Indicator diagram and noise level diagram of the engine running at 1500 R.P.M. at a brake load of 93·3 lbs /sq in. bmep with 37·1 % of the total fuel injected 36° before top dead centre Main injection 9° before top dead centre

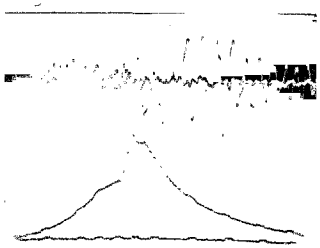


PLATE III *a*

Fuel : Kerosene

Indicator diagram and noise level diagram of the engine running at 1500 R P.M. at a brake load of 66 7 lbs /sq in. bmep (full load) without supplementary fuel injection. Main fuel injection timing 13° before top dead centre. Specific fuel consumption 0 432 lbs/bhp-hr Maximum rate of pressure rise 74.5 lbs /crank angle degree.

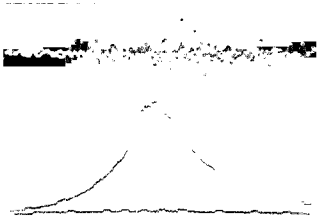


PLATE III *b*

Fuel Kerosene

Indicator diagram and noise level diagram of the engine running at 1500 R.P M. at a brake load of 66.7 lbs./sq in bmep with 59 5 % of the total fuel injected at 36° before top dead centre. Main injection timing 13° before top dead centre. Specific fuel consumption 0.431 lbs./bhp-hr. Maximum rate of pressure rise 68.6 lbs./crank angle degree

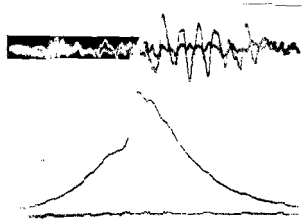


PLATE IV *a*

Fuel: Low speed diesel oil

Indicator diagram and noise level diagram of the engine running at 1500 R P M at a brake load of 66 7 lbs /sq in bmep (Full load) without supplementary fuel injection Main fuel injection 13° before top dead centre. Specific fuel consumption 0 516 lbs./Bhp-hr. Rate of pressure rise (max) 62.9 lbs crank angle degree.

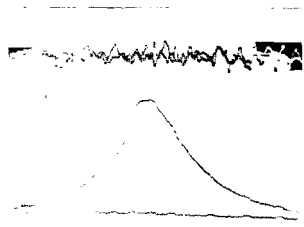


PLATE IV *b*

Fuel: Low speed diesel oil

Indicator diagrm and noise level diagram of the engine running at 1500 R P M. at a brake load of 66.7 lbs /sq in. bmep with 70 3% of the total fuel injected at 36° before top dead centre Main injection timing 13° before top dead centre Specific fuel consumption 0.497 lbs /Bhp-hr Max rate of pressure rise 60 lbs /crank angle degree.

Non-firing diagram

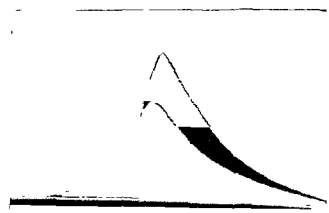


PLATE V

Pilot injection 36° B.T.D C.
Fuel rate : 0 892 lbs /hr.
Pr. increase 85 lbs /sq.in

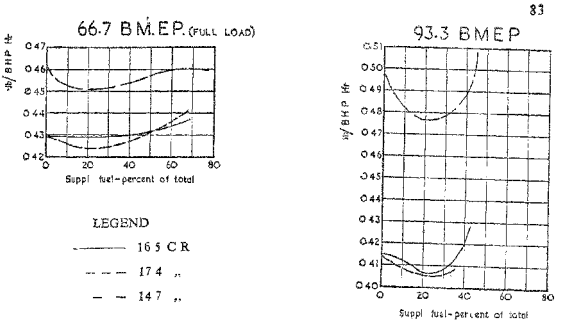


FIG XI

Main. 9° B T D C Pilot 36° B T D C

Specific fuel consumption *vs* supplementary fuel for various compression ratios

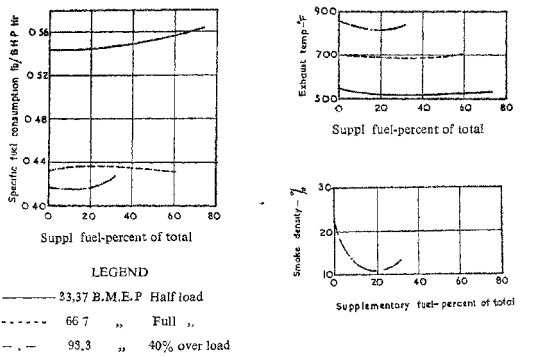
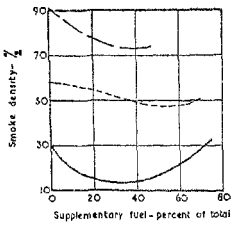
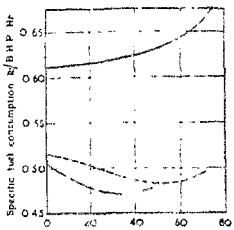
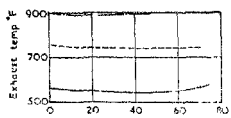


FIG XII

Fuel. Kerosene. Main: 13° B T D C Comp. ratio: 16.5 I Pilot. 36° B.T.D C







LEGEND

——— 33.35 B.M.E.P. Half load

– – – 66.7 ,, Full ,,

— . — 93 3 ,, 40% over load

FIG. XIII

Fuel : Light diesel oil. Main : 15° B.T.D.C. Comp. ratio : 16.5 : 1 Pilot · 36° B.T.D.C.

DIFFERENTIAL STAINING OF THE CELL ORGANELLES OF *ALLIUM CEPA* USING A NEW FIXATIVE AND A NEW STAIN

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Received on April 13, 1960

ABSTRACT

The reactions of the root-tip cells of *Allium cepa*, fixed in Iodine-formaldehyde-acetic acid (I.F.A.), Acetic alcohol and Navashin's fluid to Giemsa stain are compared with those obtained after staining with Methyl green—Pyronin. I. F. A. gives optimal preservation of the cell organelles but differs from Acetic alcohol in that it accentuates the blue colour of Giemsa and the red of Methyl green—Pyronin. The red colour of Giemsa is transient in material fixed in Navashin for one hour and absent in those fixed for twenty-four hours. The differential staining with Methyl green—Pyronin was observed only when the time of fixation in Navashin was limited to one hour.

The nucleus and the chromosomes in prophase and late telophases are stained purple red by Giemsa and bluish green by Methyl green—Pyronin. The chromosomes at other phases of division have a slightly darker shade. The nuclear membrane, the nucleoli, the cytoplasm, the achromatic figure and especially the phragmoplast are blue in Giemsa and red in Methyl green—Pyronin. The cell plate which is unstained in early stages of formation stains in late telophase.

INTRODUCTION

During an analysis of the reaction of the nucleus of living yeast cells to various fixatives and stains it was discovered that Iodine-formaldehyde-acetic acid (I.F.A.) solution alone gave a life-like preservation (Royan, 1956, 1958 *a*; Thyagarajan and Subramaniam, 1957; Aswathanarayana and Subramaniam, 1958). On staining the fixed but unhydrolysed smears with Giemsa's solution the yeast nucleus was shown to have a blue nuclear membrane, red chromocentres and blue nucleolar equivalents (Royan, 1958 *b*; Subramaniam *et al*, 1959). Further, the fixatives commonly employed for the study of plant nuclei did not give a life-like preservation of the organelles of the yeast nucleus (Subramaniam, 1960).

Till the demonstration of the nucleus in living yeast cells, the selection of fixatives was arbitrary. Moreover, the Giemsa stain now in vogue in yeast cytology had rarely been tested on plant material. The life-like preservation of the yeast nucleus, by I. F. A., a fixative not tried on plant material, and the facility with which the Giemsa stain could be used to stain differentially the nuclear organelles necessitated confirmation by tests on plant material.

While the utility of I. F. A. as a fixative could be evaluated in yeasts by studying the reaction of living cells with visible nuclei (Royan, 1956, 1958 *a*; Thyagarajan and Subramaniam, 1957; Aswathanarayana and Subramaniam, 1958) the same procedure would not be possible in the case of the root tips composed of several layers of cells.

A historical account of the Giemsa stain is given by Conn (1940) and its suitability for cytological investigations is indicated by Gatenby and Cowdry (1928) and Gatenby and Painter (1937). Its use in Microbial cytology is reviewed by Robinow (1944, 1956), Marshak (1955), Vendrely (1955) and Royan (1958 *a*).

Jacobson and Webb (1952) stained cells from tissue cultures in May-Grunwald followed by Giemsa and observed that the deoxyribonucleoproteins were purple red while the ribonucleoproteins were blue. Hartman and Payne (1954) obtained comparable results in *Escherichia coli* stained directly in Giemsa.

Immers (1957) employed Giemsa in his investigations on fertilization and cleavage of the sea-urchin eggs. The deoxyribonucleic acid in the pronucleus of the unfertilized sea-urchin egg was located by Agrell (1958) using Giemsa stain and enzymatic digestion. According to Kamahora, Inamori, Furusawa and Mori (1953) the constituents of the Giemsa stain have differing affinities for DNA, RNA and the proteins. The pink purple of the chromatin is said to be a mixture of the dark blue colour imparted to the DNA by Azure I and the pink colouration, by eosin, of the histone and non-histone proteins. The affinity of Methylene blue for RNA would explain the blue colour of the nucleolus and the cytoplasm.

Giemsa stain followed by a 2 % alcoholic solution of Safranin was used by Wright and Skoric (1928) to locate symbiotic bacteria in plants. In sections, the bacteria were deep blue, the cytoplasm of the plant cells light blue, the nuclei pink, the nucleoli blue and the cell walls red. This is perhaps one of the rare instances of the use of Giemsa in plant cytology. The observations embodied in this paper attempt an evaluation of the action of I. F. A. as compared to other fixatives on the root tips of *Allium cepa* and of the Giemsa stain with conventional staining methods.

MATERIAL AND METHODS

The roots of freshly germinated bulbs of *Allium cepa* were washed under the tap, excised and then transferred to the three fixatives, *viz.*, Acetic alcohol, Navashin's fluid and Iodine-formaldehyde-acetic acid. The vials were kept under an exhaust pump to ensure quick penetration and rapid fixation. Good preservation was obtained by keeping the root tips for two hours in Acetic alcohol (1 : 3) and for only an hour in Iodine-formaldehyde-acetic acid [Gram's Iodine [diluting 1 part of Lugol's Iodine (1 % I_2 in 2 % aqueous KI) with 2 parts of water] 8.8 c.c.; Formaldehyde (B. D. H. Sample, 37-41%) 1.2 c.c.; and

Glacial acetic acid (Analar quality) 0.5 c c.). Material fixed in Navashin (Darlington and La Cour, 1950, p 115) for a short period of one hour and for a longer one of 24 hours exhibited differences in their reaction to the stains. The Acetic-alcohol fixed material was down-graded while that from I.F A. was passed through two changes of 70 % alcohol extending for a period of 24 hours to remove the iodine The Navashin material was washed under the tap for 24 to 48 hours. After removal of traces of the fixatives the root tips were either stored in 70 % alcohol or immediately dehydrated, cleared in mixtures of absolute alcohol and chloroform followed by pure chloroform and then impregnated and embedded in paraffin. The blocks were sectioned at 6 μ and 10 μ.

Staining with Giemsa's Solution

The sections washed well under the tap for 15-20 minutes and kept in distilled water for five minutes were stored in Sorensen's phosphate buffer of pH 7 0 for 10 minutes. They were then transferred to the Giemsa stain (2.5 ml. of Michrome brand (Gurr) stock solution and 47.5 ml of the buffer of pH 7.0) and examined periodically up to 24 hours directly in the stain. To get the best preparations it was desirable to overstain the sections and then de-stain them carefully in 50 % alcohol prepared in buffer. After rinsing in 20 % alcohol and in buffer they were quickly dehydrated through alcohol grades, passed through alcohol-xylol (1 : 1) mixture and then through two changes of xylol before being mounted in Canada balsam. There is occasionally a shrinkage on transfer of slides from alcohol to xylol. The stain has a tendency to fade in permanent preparations. Observations were made on buffer mounts as well as from permanent preparations The former was considered necessary to evaluate the changes that occur during dehydration.

Staining with Methyl Green—Pyronin Solution

A 1 % stock solution of Methyl green—Pyronin (Gurr sample, Special for Nucleic acids) in 0 25 % Phenol in distilled water was prepared and was diluted with three times its volume of water just before use. Slides rinsed in distilled water were stored in it for 15 to 30 min. They were then rinsed in distilled water, blotted to remove the excess of water and passed through absolute alcohol, absolute alcohol-xylol mixture (1 : 1), and three changes of xylol before mounting in Canada balsam. The overstaining, if any, was removed by absolute alcohol.

Observations were made with a Bausch and Lomb Research Microscope using a × 90 objective and a × 10 eye-piece and the selected stages were photographed at 1/3 their magnification on Kodak Microfile film using a Leica attachment. Colour transparencies of some of the stages were prepared on Kodak Ektachrome film.

OBSERVATIONS

1. *Acetic alcohol—Giemsa* :—The progress of staining on transfer of sections to Giemsa solution was followed. After 5 to 15 minutes in the stain, the nuclei and chromosomes were greenish blue, while the other organelles were blue. On continued stay the blue began to be more prominent and the nuclei and chromosomes slowly stood out as deep purple structures.

Attention is invited to the fact that the red component of the Giemsa stain begins to have an affinity for the structures only when the cytoplasm has become deep blue.

When the sections were examined after removal from the bath and without rinsing, the blue colour dominated. The nuclei were purplish red. Differentiation in alcohol removes the excess of blue and leaves the nuclear matrix purple red. If this process is continued the purple red staining is also removed by alcohol leaving the nucleus greenish blue (Subramanyam and Subramaniam, 1957, Photo 1) Direct examination in the stain does not reveal the nuclear envelope owing to the intense staining of the nucleus. It is only when the nucleus is purple red after differentiation, that the blue nuclear membrane could be recognised (Photo 1). The slides were therefore differentiated in alcohol to give the desired grade. When on prolonged differentiation in alcohol the nucleus is greenish blue, the nuclear membrane still retained the blue staining (Photo 23, Subramaniam *et al*, 1959). The nucleoli not discernible in overstained nuclei became clear on differentiation in alcohol. They appeared as bluish organelles (Photos 1 and 2) Over-differentiation improved the clarity of the nucleoli which appeared as if they had stained borders. The cytoplasm was generally blue.

During prophase and late telophase the chromosomes were purple red (Photos 2 and 8). In prometa-(Photo 3), meta-(Photo 4) and anaphases (Photos 5 and 6) the chromosomes were dark owing to a coupling of the purplish red with blue In early telophase (Photo 7) the chromosomes were deep purplish red especially when they were contracting at the poles. The polar caps in prophase (Photo 2), the developing (Photo 3), and the fully formed spindle at metaphase (Photo 4), the interzonal fibres and other regions of the spindle in anaphase (Photos 5 and 6) and the phragmoplast in early telophase (Photo 7) were stained blue. This was particularly prominent in the phragmoplast. The achromatic figure, though of the same colour as the cytoplasm, stood out owing to its greater affinity for the blue component. The cell plate seen in early telophase as an unstained streak (Photo 7) began to stain blue in late telophase along with the two knob-like remnants of the phragmoplast at its end (Photo 8).

In using Giemsa as a regressive stain the time of stay in the staining bath necessary to give good preparations was also explored. Acetic alcohol material stained for one hour gave good preparations on differentiation. A longer stay in the staining bath naturally necessitated a longer time of de-staining.

Unlike the earlier workers who appear to have used Giemsa stain as a progressive one (Jacobson and Webb, 1952) the observations reported above are based on the results obtained by differentiating overstained sections. It became interesting therefore to evaluate the utility of Giemsa as compared to Methyl green-Pyronin.

2. *Acetic alcohol—Methyl green-Pyronin* —Shimamura and Ota (1956) offer evidence for the presence of pentose nucleic acid in the achromatic figure. They record the absence of pentose nucleic acid in the cell plate, which appeared as an unstained line in the middle of the phragmoplast. Their conclusions are drawn from sections differentially stained with Toluidine blue, Thionin and Methyl green-Pyronin. The fixatives used were Acetic alcohol and Telyesniczky's fluid

On staining sections of *Allium cepa*, fixed in Acetic alcohol, the nuclear membrane (Photo 16) and the nucleoli (Photos 16 and 17) were red, while the nuclear matrix showed bluish green areas (Photo 16). The chromosomes in prophase (Photo 17), as well as late telophase (Photo 22) were bluish green, while in prometa-(Photo 18), meta-(Photo 19), ana-(Photo 20) and early telophases (Photo 21) they tended to be more bluish The polar caps (Photo 17), the developing spindle in prometaphase (Photo 18), the spindle at metaphase (Photo 19), the poleward regions of the spindle (Photo 20), the phragmoplast (Photo 21) and its knob-like remnants and the cell plate (Photo 22) were all stained red. It is only during the early telophase (Photo 21) that the cell plate remained unstained, reminiscent of a similar experience with the Giemsa stain (Photo 7).

3. *Iodine-formaldehyde-acetic acid—Giemsa* '—The reaction of the cell organelles to Giemsa in material fixed in I F.A. was comparable to that observed after Acetic alcohol fixation. Whereas after Acetic alcohol the chromosomes and nuclei assumed a purplish red tinge after a stay of 30 to 45 minutes in the stain, in I F A. material the identical organelles became purplish red only after a lapse of one to three hours. The blue colour of the cytoplasm appeared more intense when the sections were examined directly from the stain. But this could be removed easily during differentiation. In permanent preparations the organelles having an affinity for the blue component had a more intense colour as compared to those of cells fixed in Acetic alcohol

When material over-stained in Giemsa was carefully differentiated in 50% alcohol, the nuclear matrix was purplish red, the nucleoli and the nuclear membrane blue (Photo 9) The chromosomes in prophase (Photo 10) and and late telophase (Photo 15) were purple red. But this colour appeared deeper in prometa-(Photo 11), meta-(Photo 12), ana-(Photo 13) and early telophases (Photo 14) The cytoplasm and the achromatic figure (Photos 11 to 13) were stained in shades of blue The cell plate (Photo 14) appeared unstained in early telophase The spindle fibres and especially the interzonal

ones were clear in Acetic alcohol as well as in I F. A. Giemsa preparations (Photos 6 and 13).

4 *Iodine-formaldehyde-acetic acid—Methyl green-Pyronin*.—The nuclear membrane and the nucleoli were stained red (Photos 23 and 24). The chromatin of the resting nuclei (Photo 23) and the chromosomes at prophase (Photo 24) and late telophase (Photo 29) were stained bluish green. The chromosomes during prometa- (Photo 25), meta- (Photo 26), ana- (Photo 27) and early telophases (Photo 28) tended more towards blue. The polar caps (Photo 24), the spindle (Photos 25 and 26), the poleward and the interzonal regions of the spindle at anaphase (Photo 27) and the phragmoplast (Photo 28) were stained red. The cell plate seen as an unstained area in early telophase (Photo 28) appeared as a red line in late telophase (Photo 29) along with the remnants of the phragmoplast at its sides. Material fixed in I. F. A. had a greater affinity for the red colour of Methyl green-Pyronin. Attention was more concentrated on the contrast of the cell organelles in order to evaluate whether Giemsa gave as specific a distinction between the chromosomes and the achromatic figure as Methyl green-Pyronin.

5. *Navashin—Giemsa*:—Unlike the two previous fixatives the cell organelles were not stained in differential colours by Giemsa in material fixed for 24 hours in Navashin's fluid. All the organelles though clear appeared in different shades of blue. The cell plate was seen only as a stained line on the equator of the phragmoplast. The characteristic red or purplish red staining of the chromosomes was not observed either when over-stained slides were examined directly in the stain or during de-staining in 50% alcohol.

Such an experience led to a doubt whether the absence of affinity for the red component of the Giemsa stain may not be due to over-fixation. Therefore, the reaction of the cells to Giemsa after fixation for an hour in Navashin's fluid was tested. Direct examination in the stain showed the dark blue nuclei and the chromosomes being overlaid by a slight reddish tinge after a stay in Giemsa's solution for one to three hours. The red tinge was very transient even in wet preparations and disappeared completely during the quick dehydration necessary for making the slides permanent.

6. *Navashin—Methyl green-Pyronin*:—Contrary to the experience with Giemsa where the red colour was transient, a positive differential staining with Methyl green-Pyronin was obtained when the fixation time in Navashin had been limited to one hour.

The chromatin in Photo 30 was bluish green. The nuclear boundary, the nucleoli (Photos 30 and 31), the cytoplasm, the polar caps (Photo 31), the spindle (Photos 32 to 34), the phragmoplast (Photos 35 and 36) and the cell plate with the remnants of the phragmoplast (Photo 37) were all stained in a red colour. The cell plate was seen as an unstained line in early telophase

(Photo 35) only in a few instances In many, the cell plate was stained red both in early as well as late telophases (Photos 36 and 37).

The difficulty experienced in getting a differential staining of the cell organelles with Giemsa in material fixed in Navashin for 24 hours was parallelled by the experience in attempts to stain such sections with Methyl green-Pyronin. Even after storage for 24 hours the nuclei and chromosomes were only red. The bluish green colour assumed by the chromatin when Acetic alcohol or I. F. A. fixed material were stained with Methyl green-Pyronin was absent in Navashin material fixed for 24 hours.

The reaction of the various organelles to staining with Giemsa and Methyl green-Pyronin solutions are summarized in Table I.

TABLE I

STAINING REACTIONS

Organelles	*Giemsa*	*Methyl green-Pyronin*
I. *Resting cells*		
(i) Chromatin	Purple red	Bluish green
(ii) Nuclear membrane (iii) Nucleolus (iv) Cytoplasm	Blue	Red
II. *Dividing cells*		
(i) Chromosomes		
(*a*) Prophase and late telophase	Purple red	Bluish green
(*b*) Meta-, ana-, and early telophases	Dark Purplish red (Purple red + blue)	Dark Bluish green
(ii) Polar caps (iii) Spindle (iv) Poleward regions of the spindle (v) Phragmoplast	Blue	Red
(vi) Cell Plate		
(*a*) Early telophase	Unstained	Unstained
(*b*) Late telophase	Stained	Stained

DISCUSSION

Iodine-formaldehyde-acetic acid gave optimal preservation of the cell organelles. Material fixed in it can be sectioned with ease unlike those in Acetic alcohol which show shrinkage as well as brittleness. The difference between I. F. A and Acetic alcohol is that there is an accentuation of the blue colour of Giemsa and the red of Methyl green-Pyronin in the former fixative.

Immers (1957) recorded his inability to stain the resting nuclei during the first mitosis of the fertilized sea-urchin eggs The nuclei of the root tip cells of *Allium cepa*, however, show a clear differential staining (Photos 1 and 9). Jacobson and Webb (1952) described the nuclear membrane as colourless while Agrell (1958) reported it as faint red in the pronucleus of sea-urchin eggs In *Allium cepa* the nuclear membrane has the same colour as the cytoplasm and the nucleolus (Photos 1 and 9; *c f* Subramanyam and Subramaniam, 1957, Photo 1). This is reminiscent of the staining of nuclear membrane red (Photos 16, 23 and 30) by Methyl green-Pyronin and would conform to the presence of a distinct membrane reported from electron micrographs of plant (De, 1957) and animal cells (Callan and Tomlin, 1950; Watson, 1954; Kautz and De Marsh, 1955; and Barer *et al*, 1959)

The slight variations in the colour of the chromosomes during the various phases of division in *Allium cepa* (Table 1), parallel a similar observation by Jacobson and Webb (1952) and disagree with those of Immers (1957) who records a uniform magenta tint during all the phases.

Jacobson and Webb (1952) suggested that the deoxyribonucleoproteins were stained purple red by May-Grunwald-Giemsa and the ribonucleoproteins blue (*c f.* Kamahora *et al*, 1953). Confirmation for the above was offered by them by staining isolated nucleoproteins before and after treatment with enzymes The nucleoli and the cytoplasm of *Allium* which are blue in Giemsa (Photos 1, 2, 9 and 10) are red in Methyl green-Pyronin (Photos 16, 17, 23, 24, 30 and 31; *See* Table I) It should be emphasized, however, that the composition of the fixative and the time of fixation are major factors in obtaining a polychromatic staining This is evident from the transient nature of the red colour of Giemsa in Navashin material and an accentuation of the blue colour of Giemsa and the red of Methyl green-Pyronin in I. F. A. material.

Plant cells fixed in Acetic alcohol and stained with Giemsa showed the same differential staining of the nuclear organelles as in yeast cells fixed in I F. A. (Subramaniam *et al.* 1959). Evidence is presented in this paper to show that this similarity in the structure of yeast and plant nuclei could be demonstrated in material fixed in I. F. A and stained with Giemsa

ACKNOWLEDGEMENT

Grateful acknowledgement is made to Dr. M K. Subramaniam for his guidance and encouragement and to the authorities of the Indian Institute of Science, Bangalore, for the award of a Scholarship.

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DESCRIPTION OF PHOTOMICROGRAPHS

PLATE I

Photos 1-8 Sections of roots fixed in Acetic alcohol and stained with Giemsa × ca., 2,000.

1 The nuclear membrane is continuous

2 Prophase The polar caps merge with the nuclear membrane

3 Prometaphase The fibrous nature of the spindle is clear

4 Metaphase The prominently stained chromosomes lie on the equator of the spindle

5 Anaphase The poleward regions of the spindle are very clear

6 Anaphase The interzonal fibres are blue

7 Early telophase The phragmoplast is seen with the clear unstained cell plate.

8 Late telophase The cell plate shows the remnants of the phragmoplast at its ends

9-12 Sections of the root tips fixed in Iodine-formaldehyde-acetic acid and stained with Giemsa × ca, 2,000

9 The nuclear membrane is clear

10 Prophase. The polar caps and the nuclear membrane are discernible

11 Prometaphase The fibrous spindle is very distinct

12 Metaphase The spindle and the chromosomes are clear

PLATE II

13-15 Sections of roots fixed in Iodine-formaldehyde-acetic acid and stained with Giemsa ×ca., 2,000

13 Anaphase with the poleward and interzonal regions of the spindle

14 Early telophase. The phragmoplast is prominent. The cell plate is unstained

15 Late telophase with the stained cell plate and the knob-like remnants of the phragmoplast

16-22 Sections of root tips fixed in Acetic alcohol and stained with Methyl green-Pyronin. × ca, 2,000

16 Resting nucleus The two nucleoli and the nuclear membrane are stained red.

17 Prophase with polar caps imperceptibly merging with the nuclear membrane.

18 The fibrous spindle stained red is clear

19 Metaphase with the spindle.

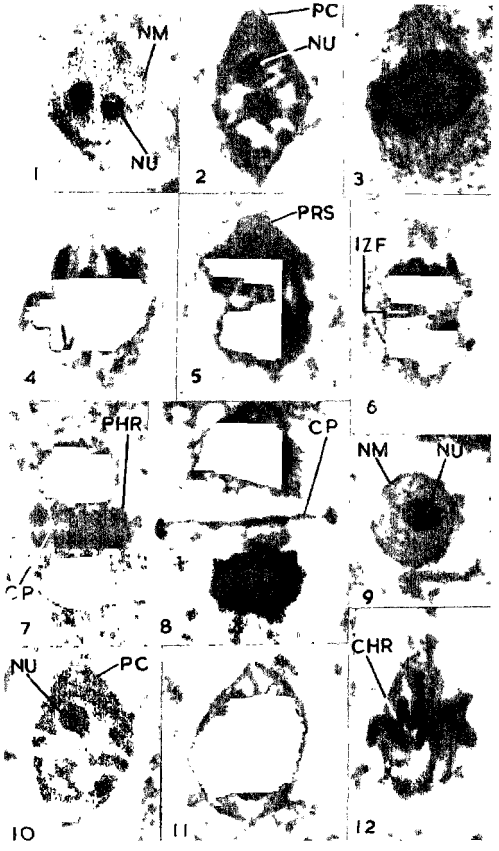

PLATE I

Differential staining of the cell organelles of *Allium cepa* using a new fixative and a new stain

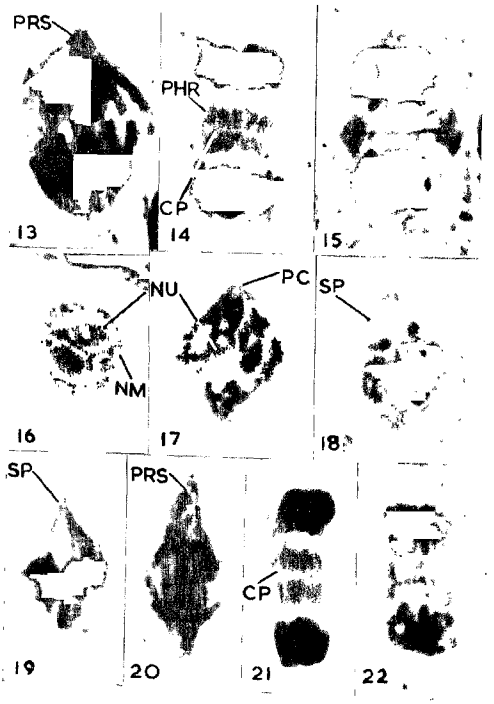

PLATE II
Differential staining of the cell organelles of *Allium cepa* using a new fixative and a new stain

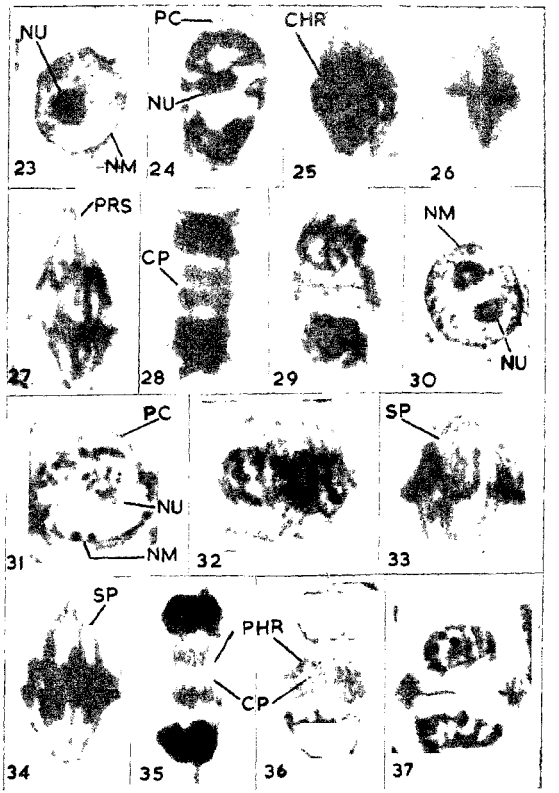

PLATE III

Differential staining of the cell organelles of *Allium cepa* using a new fixative and a new stain

20 The poleward regions and the interzonal part are distinct

21 Early telophase The unstained cell plate is seen along the middle of the phragmoplast

22 Late telophase showing the stained cell plate

PLATE III

23-29 Sections of roots fixed in Iodine-formaldehyde-acetic acid and stained with Methyl green-Pyronin × ca , 2,000

23 The nuclear membrane is as thick as in Giemsa preparations

24 Note the contrast between the polar caps and the chromosomes

25 Prometaphase The chromosomes are stained darker

26 Metaphase The spindle is obscuring the chromosomes

27 Anaphase The interzonal and the poleward regions are stained in almost the same intensity

28 Early telophase Note the unstained cell plate on the equator of the phragmoplast

29 Late telophase The cell plate and the remnants of the phragmoplast are stained

30-37 Sections of roots fixed in Navashin's fluid and stained with Methyl green-Pyronin × ca , 2,000

30 Note the nuclear membrane.

31 Prophase The polar cap is outside the nuclear membrane

32 Prometaphase The fibrous spindle can be seen distinctly

33 Metaphase Note the spindle fibres converging at the poles.

34 Early anaphase with the bipolar spindle converging at both the poles

35 Early telophase. Phragmoplast with the unstained cell plate

36 Early telophase Phragmoplast with the stained cell plate

37 Late telophase The remnants of the phrgmoplast are prominent

KEY TO LETTERING

CP , Cell Plate, CHR , Chromosomes , IZF., Interzonal fibres ; NM , Nuclear membrane; NU , Nucleolus ; PHR., Phragmoplast , PC , Polar cap ; PRS , Poleward regions of the spindle ; SP , Spindle